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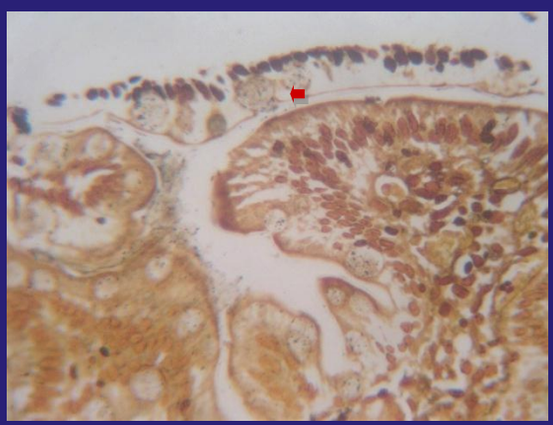

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Correlation Between MTB/RIF Gene Xpert Cycle Threshold Values and Clinical Radiological Severity of Pulmonary Tuberculosis

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Abstract

The determination of bacterial load is essential for assessing disease severity, transmission rate, and prognosis. GeneXpert is a diagnostic test that provides Cycle Threshold (Ct) value as a potential measure of *Mycobacterium Tuberculosis* (Mtb) load. Despite its potential, there are limited reports exploring the relationship between Ct value and clinicoradiologial severity. This study aimed to correlate Ct value and clinicoradiological severity of pulmonary tuberculosis (TB). The study was a retrospective design using medical record data of confirmed TB patients from January to December 2022. These patients were identified based on GeneXpert test and classified as high, moderate, or low detection Mtb when Ct value was <16, 16-22, and 22-28, respectively. In assessing the severity of clinical using the Bandim score, thoracic TB lesions were categorized by chest X-ray into minimal, moderate, and advanced. Of the total of 90 TB patients the majority were males (78.9%) aged 46-65 years (59.0%), with comorbidities (95.0%). Most of the participants had mild clinical severity (44.4%), with Ct value of 16-22 (52.2%), and moderate lesions (35.6%). The most common lesions were fibroinfiltrates on the chest x-ray (61.1%). The Ct value of <16 had a significant correlation with clinical severity of TB ($p<0.05$) but no significant association with advanced lesions ($p>0.05$). Based on the results, Ct value had a strong correlation with clinical severity in pulmonary TB. In addition, it could be used as a predictor for managing pulmonary TB patients and an important indicator for control programs.

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INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb), which can be prevented and cured.[1] It is also a heavy burden in several Asian countries, particularly India, recognized as the biggest nation in Asia's southeast. Several studies have shown that India has the highest TB prevalence, with 210 occurrences per 100,000 people in 2021 as the yearly rate. Meanwhile, Indonesia has the third highest incidence of TB, having a yearly rate of 75.2% of pulmonary TB occurrences in 2021.[2] The gold standard for establishing a diagnosis of this disease is dependent on various microbiological tests, each with its unique advantages and limitations. Although microscopy has been reported to have high specificity, it is largely limited by low sensitivity. Applying culture to liquid and solid substances is an alternate method for identifying TB, but this method often causes delays in detection (6-8 weeks). According to previous studies, liquid media for culture, like the BacT/ALERT system, Mycobacterial Growth Indicator Tube (MGIT), Microscopic Observation Drug Susceptibility Assay (MODS), and Bactec 460TB system have the potential to offer faster results within 7 to 10 days. However, culture test is more expensive and requires subculture on solid media for optimization.[3] To overcome these limitations, several reports have proposed the use of Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), which is a computerized real-time genetic assay to identify rifampicin resistance and diagnose tuberculosis quickly. This assay is advised to be used as a first examination for people who may have multidrug-resistant tuberculosis (MDR-TB) or TB correlated with the human immunodeficiency virus.[4]

In 2013, the WHO issued a recommendation advocating for the adoption of Xpert as the first diagnostic examination among every adult and kids exhibiting TB symptoms.[4] The GeneXpert test uses advanced molecular technology to detect duplicated DNA sequences through real-time nested-Polymerase Chain Reaction (Rt-PCR) assays. In addition, the resultant Ct value serves as a semiquantitative measure, offering insight into the number of TB bacilli in sputum samples.[5] This test correlates Ct value with the number of acid-resistant bacilli observed through microscopy and the time required to produce a positive culture of Mtb using liquid culture system. Despite its potential, few researches have explored the correlation between Ct value and various clinical characteristics, such as the severity of TB, demographic of TB patient, severity of lung parenchymal damage, and drug resistance.

The Ct value in pulmonary TB plays a role in determining bacterial load, replication patterns, and patient prognosis.[6] A cohort study analyzed the relationship between the parameter and the clinical outcomes of pulmonary TB patients. The results showed that the lower the Ct value or the fewer the PCR machine rounds, the worse the clinical outcome due to higher replication and rapidly increasing bacterial load.[7] The parameter is typically categorized into high (22-28), medium (16-22), and low (<16) ranges, indicating varying levels of detectable Mtb concentration. The continuous variable Ct value has an inverse relationship with the quantity of Mtb that is present. In molecular rapid tests, it represents the number of times the PCR machine is spun to determine the presence of bacteria, where more spins indicate fewer bacteria, and vice versa. Low Ct value indicates that the machine rotates only a few times and the bacterial load is high due to high bacterial replication. The real-time Reverse Transcription - Polymerase Chain

Reaction (RT-PCR) Ct value represents the number of amplification cycles and is inversely related to viral load. The parameter can also be used semi-quantitatively to predict viral load, which offers valuable insights into disease prognosis.[8]

Several reports have discussed the correlation between GeneXpert and other TB screening modalities,[9,10] but there are limited studies analyzing the Ct value with clinical profiles and TB severity in pulmonary tuberculosis patients. Therefore, this study aimed to evaluate the correlation between the Ct value and the clinical profile and degree of severity pulmonary tuberculosis.

MATERIALS AND METHODS

Methods

This was a retrospective study, which was carried out based on patients' medical record data at the TB service center of Dr. Zainoel Abidin Hospital Banda Aceh from January 2020 to July 2021, for 19 months, with a total of 106 participants. The medical records were obtained.

Inclusion Criteria

Patients fulfilled the inclusion requirements with pulmonary TB based on GeneXpert MTB/RIF examination and had complete medical record data. In addition, eligible participants were aged ≥18 years with cough symptoms lasting more than two weeks, accompanied by sweating at night, loss of weight, shortness of breath, a high temperature, chest discomfort, and bloody cough.

Exclusion Criteria

Patients diagnosed with extra-pulmonary TB and patients diagnosed with drug-resistant pulmonary TB (DR-TB) were excluded from the study.

Sample Collection

Clinical data such as cough, sweating at night, loss of weight, shortness of breath, high temperature, chest discomfort, and bloody cough were then inputted for analysis and categorized into mild, moderate, and severe groups based on the Bandim TB score. It was based on these signs, symptoms, and indicators, such as tachycardia, anemia conjunctiva, positive lung auscultation findings, axillary temperature >37.0°C, and body mass index (BMI) of <18 in addition to clinical abnormalities.[11] Upper Mid Arm Circumference was not inputted as the data were not present in the medical records. Details of each patient's data were recorded in the data collection sheet. Ct values from GeneXpert were categorized as high, medium, and low. Mtb was deemed identified when a positive response with a Ct value of ≤38 was observed in at least two of the five probes. Semiquantitative estimation of the Bacilli concentration was performed using the Ct 22–28 range in the high category, indicating low detectable Mtb, Ct 16-22 in the medium category, indicating medium detectable Mtb, and Ct <16 in the low category, showing high detectable MTB.[8] In the assessment of chest X-ray, all radiologic lesions found on thoracic photographs, such as consolidation, nodular opacity, and cavities, were entered into the data collection sheet. Pleural effusion, pneumothorax, fibrosis, and hilar lymphadenopathy were also observed. The degree of disease extent based on chest X-ray was divided into (1) Minimal degree when the fibroinfiltrate lesion was slight with no demonstrable cavities, no change in lung volume, only above the second chondrosternal junction, and at the level of the fourth thoracic vertebra. (2) Moderate degree when the diffuse fibroinfiltrate lesion had moderate density and could extend to the entire lung on one side, there was a

cavity with a diameter of less than 4 cm. (3) Advanced degree, where the lesion was more extensive than the moderate degree.[12]

Data Analysis

Statistical indicators like frequency and percentage were computed for qualitative information, whereas mean and standard deviation (SD) were determined for quantitative data. Kruskal Wallis test was used to assess the association of Ct value with the clinical severity of pulmonary TB and the severity of radiology, which was considered significant when the p-value <0.05. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 23.0.

RESULTS AND DISCUSSION

Among the 90 patients with pulmonary TB in this study, 78.9% were males, 59% were aged 46-65 years, and 95.0% had comorbidities, with varying types, such as type 2 diabetes (29%), malnutrition (55%), hypertension (12%), heart disease (6%), and kidney function abnormalities (3%). The majority of pulmonary TB patients had mild clinical severity (44.4%), with clinical complaints of cough (94.4%), shortness of breath (52.2%), chest pain (57.8%), and anemia (83.3%). In addition, most of the participants showed medium Ct value (52.2%), chest X-ray with moderate lesion (35.6%), and decreased hemoglobin levels (83.3%), as shown in Table 1.

Table 1. Clinical characteristics of study participants.

Characteristics	Frequency (n)	Percentage (%)
Gender		
Male	71	78.9
Female	19	21.1
Age		
18-45 years	39	43.3
46-65 years	51	56.7
Comorbidities		
With comorbid	85	85.0
Without comorbid	5	5.0
Comorbid		
DM type 2	23	25.6
Malnutrition	50	55.6
Hypertension	11	12.2
Heart Disease	5	5.6
Acute Kidney Injury	3	3.3
Clinical symptoms		
Cough	85	94.4
Hemoptysis	24	26.7
Temperature >37°C	31	34.4
Night Sweats	23	25.6
Shortness of breath	47	52.2
Chest Pain	52	57.8
Abnormal Auscultation	43	47.8
Heart Rate >90	48	53.3
BMI <18	39	43.3
BMI <16	15	16.7
Anemia	75	83.3
TB severity (Bandim score)		
Mild degree	40	44.4
Moderate Degree	36	40.0
Severe Degree	14	15.6
GeneXpert		
High	19	21.1
Medium	47	52.2
Low	24	26.7
CT value Xpert		
Low	19	21.1
Medium	47	52.2
High	24	26.7
Hemoglobin Level		
Normal	15	16.7
Decreased	75	83.3
Chest X-Ray		
Minimal	17	18.9
Moderate	41	45.6
Advanced	32	35.6

The most common chest X-ray lesion shown in this study was fibroinfiltrate at 61.1%, followed by consolidation (23.3%), pleural effusion (16.7%), and pneumothorax (6%). Meanwhile, pneumothorax was not a typical TB lesion but could be secondary to Mtb infection, as shown in Figure 1.

Table 3. Correlation of GeneXpert Ct value with clinical radiological severity of TB patients

Variable	CT value GeneXpert						Total		OR (CI 95%)	p-value
	High		Medium		Low					
	n	%	n	%	n	%	n	%		
Clinical Severity										
Mild	12	30.0	24	60.0	4	10.0	40	100.0	1.357 (0.532-3.465)	0.006
Moderate	11	30.6	18	50.0	7	19.4	36	100.0		
Severe	1	7.1	5	35.7	8	57.1	14	100.0		
Chest X-Ray Severity										
Minimal	5	29.4	11	64.7	1	5.9	17	100.0	1.184 (0.369-3.804)	0.145
Medium	7	17.1	23	56.1	11	26.8	41	100.0		
Advanced	12	37.5	13	40.6	7	21.9	32	100.0		

Low Ct value examination had a significant correlation with severe clinical degree, while high Ct value had a significant association with mild clinical degree of pulmonary TB patients ($p<0.05$). Meanwhile, Table 3 indicates that there was no statistically significant correlation between Ct value and TB lesions based on chest X-ray.

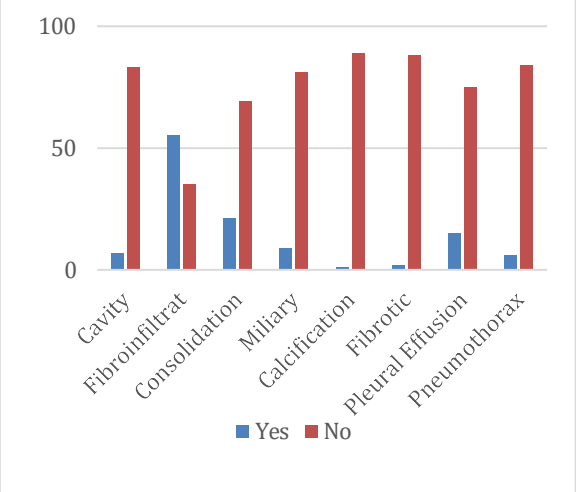

Figure 1. Distribution of typical TB lesions based on chest X-ray.

Receiver Operating Curve (ROC) analysis was used to assess predictors of pulmonary TB severity through Ct value. The results showed that the medium Ct value (C_T 16-22) could be used as a predictor of pulmonary TB severity with a sensitivity value of 92.9% and specificity of 30.3% ($p = 0.003$) (Figure 2).

The majority of pulmonary TB patients in this study were males, accounting for 78.0% of the total population, as shown in Table 1. This finding was supported by a meta-analysis showing that males were the predominant TB patients in low- and middle-income countries, with a male-to-female ratio of 2.21: 1.[13] According to the WHO, pulmonary TB cases in males are 1.8 times higher compared to females.[14] The majority of men as the characteristic findings of this study are similar to findings by Noviyani et al.[15]

The higher prevalence could possibly be caused by better access to health services[13]. Males were more susceptible to infection or at risk of exposure, and a higher prevalence of smoking was correlated with toxic lung injury, leading to decreased immune cell function and increased susceptibility.[14,16]

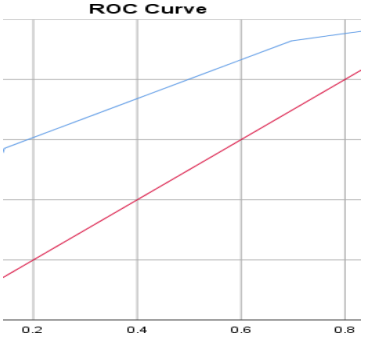

Figure 2. ROC curve (blue line) of Ct value on clinical severity of pulmonary TB.

Of the participants in the present research, 59.0% were between the ages of 46 and 65 of the total population, as shown in Table 1. Previous studies related to the occurrence of TB in Indonesia found that the highest prevalence was found among individuals aged ≥ 55 years.[15] The susceptibility of elderly individuals to TB infection correlated with increased vulnerability to infection and reactivation of Mtb infection. In addition, as people age, the lung's cellular and physiological alterations coincide, resulting in a low-grade oxidative state that is pro-inflammatory and persistent.[17] As a result, the stress response's homeostatic balance was upset, which increased the risk of oxidative stress, mitochondrial dysfunction, cell damage, decreased lung function, impaired immunosurveillance, and an increased vulnerability to respiratory and chronic illnesses like tuberculosis. These unfavorable changes were primarily brought on by inflammation and immuno-senescence. Decreased immunity, comorbidities, and its vulnerability to harmful medication side effects, among other things, puts aged people at an increased risk of tuberculosis (TB) illness and death.[18,19]

Based on the results, the most common thoracic photo findings found in samples with pulmonary TB were fibroinfiltrates at 61.1%, followed by consolidation at 23.3% (Figure 1). Furthermore, fibroinfiltrates were a combination of fibrosis and infiltrates. Fibrosis was caused by long-term lung tissue damage characterized by an abundance of extracellular matrix deposits in the airways, replacing typical lung parenchyma containing collagenous material that caused changes in structure, such as the lung wall's thickness and stiffness.[20] A study by Majdawati showed that 66% of patients showed infiltrate lesions, 36% had combined lesions, 8% had fibroinfiltrates, and 90% had no lesions. Infiltrate lesions and cavities unevenly distributed on chest X-ray were more often found in pulmonary TB cases with Mtb positive sputum results compared to sputum-negative cases. Infiltrates and cavities were the most common lesions found in patients with pulmonary TB.[21]

This study showed a significant correlation of Ct value with clinical severity of pulmonary TB, as shown in Table 3. The lower the Ct value, the more severe the clinical severity of patients with the condition. The medium Ct value (C_T 16-22) could be used to predict pulmonary TB severity with a sensitivity value of 92.9% and specificity of 30.3% (figure 2). Several studies had reported that GeneXpert examination played a role in determining bacterial replication by measuring Ct value and dividing Ct results into 3 groups, namely high <16, medium 16-22, and low 22-28.[22] Ct quantitatively described the number of mycobacteria, which was inversely related to the concentration of TB bacilli. A high Ct value indicated a high number of bacilli/bacterial load.[23] In line with previous reports, bacterial load was an early marker of TB infection severity. Furthermore, it played a role in the transmission of infection and propagation of mycobacterium as well as an important virulence factor. The findings showed that Ct value with high bacterial load had a significant correlation with low pulmonary TB severity. Studies that directly assessed the relationship between bacterial load and clinical severity of pulmonary TB were limited. Various references indicated the superiority of molecular testing using this PCR method to conventional sputum examination. Several studies have also compared the Ct value with others and investigated the relationship between the Ct category and its response to positivity

and cultured positivity using smear microscopy. A report assessing the Ct value of Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) with TB severity in India showed the absence of a correlation between the Ct value of CBNAAT and clinical profile of pulmonary TB, possibly due to the small number of samples.[8,24] Another study exploring bacterial load and inflammatory reaction with clinical severity in pneumonia showed that increased bacterial load correlated with manifestations of high fever (>39.1°C). In addition, low Ct value correlated with more invasive bacterial activity and complications of pneumonia.[25]

STRENGTH AND LIMITATION

This investigation was restricted to clinical observations made after the fact using patient medical record data. Moreover, it was conducted in a single study center and did not include a control group. Therefore, various places need to be studied in order to produce a validated result for more exact and accurate results as well as greater generality of the findings.

CONCLUSIONS

In conclusion, Ct value had a significant correlation with pulmonary TB. In addition, the diagnostic accuracy set at medium Ct value (CT 16-22) could predict pulmonary TB severity. Based on these findings, Ct value could be used as a predictor for managing pulmonary TB patients and an important indicator for TB control programs.

ETHICAL CLEARANCE

The research protocol was approved by Ethics Committee Approval from the Organizational Ethics Committee (Note Number 146/ETIK-RSUDZA/2023).

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Sponsorship was not provided for this study.

CONFLICT OF INTEREST

There is absolutely no conflict of interest with this study.

AUTHOR CONTRIBUTION

The study was designed by BY and RM. Reviewing the literature was done by YA. With collaboration from all authors, BY and YA analyzed the statistical data, RM prepared the paper, and RM collected data in the clinics.

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Original Article

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Comparative Analysis of Essential Oil Profiles From Emprit Ginger Rhizome (*Zingiber officinale* var. amarum) Grown in Different Locations and Antibacterial Activity Againts *Staphylococcus aureus*

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Chemometrics

Abstract

Emprit ginger (*Zingiber officinale* var. Amarum) is a native Indonesian medicinal plant used to treat various diseases. Apart from being an antioxidant, ginger emprit also has antibacterial potential. However, herbal materials used for medicinal purposes produce inconsistent effects due to the fluctuating chemical composition of the plants, usually caused by differences in growing locations that affect the content of active metabolites. This study aims to evaluate the antibacterial activity of essential oil of emprit ginger rhizome. Samples were obtained from 14 different growing locations namely Ponorogo, Magetan, Pacitan, Wonogiri, Karanganyar, Boyolali, Semarang, Magelang, Purworejo, Temanggung, Wonosobo, Banyumas, Bantul, and Kulonprogo. The essential oil profile of emprit ginger was obtained through Gas Chromathography Mass Spectometry (GCMS). Analyzed by multivariate calibration of *Principal Component Analysis* (PCA) and *Orthogonal Partial Least Squares* (OPLS) using SIMCA *software*. Antibacterial activity of essential oils was performed by microdilution method against *Staphylococcus aureus* bacteria. Analysis of antibacterial activity was determined by probit method to obtain the Minimum Inhibitory Concentration-50 (MIC_{50}). The results of the GCMS spectrum of essential oil showed that the main compound components in ginger emprit essential oil are *Citral, Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl, exo-(CAS), Z-Citral, Geranyl acetate, Camphene, 1,6 Octadiene, 7 Methyl-3-Metylene, 1,6-Cineole, Farnesene, Bornylene, Beta-Myrcene, Zingiberene, and Alpha-pinene*. The results of the *Staphylococcus aureus* antibacterial activity test on emprit ginger rhizome essential oil from Boyolali area show the highest MIC_{50} value of 0.2011% v/v.

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INTRODUCTION

Indonesia has plant species that are scattered in various regions, where the existing biodiversity can be utilized as medicinal raw materials. Indonesian people have long recognized and used traditional medicine to treat various diseases. One plant that is often used by the community is ginger (*Zingiber officinale* Roscoe), which is one of the spices in the temu-temuan tribe (*Zingiberaceae*).

Ginger is a medicinal plant and spice characterized by its pseudostem structure, scientifically known as *Zingiber officinale* var. Amarum. The variant known as emprit ginger is a rhizome plant that grows in low-lying to mountainous regions at altitudes ranging from sea level to 1500 meters.[1] Ginger originates from the Pacific Asia region, predominantly from India to China. As such, these two nations are often recognized as the first to utilize ginger, primarily as a beverage ingredient, cooking spice, and in traditional medicine. The largest global concentrations of ginger plants are in tropical regions, particularly across Asia and the Pacific Islands. Cultivation has recently extended to Jamaica, Brazil, Hawaii, Africa, India, China, Japan, the Philippines, Australia, New Zealand, Thailand, and Indonesia. In Indonesia, ginger is found throughout the country, grown in both monoculture and polyculture systems.[2] Based on the form, color, and size of the rhizomes, three types of ginger are recognized: large white ginger (also known as rhino ginger), small white ginger (or emprit ginger), and sunti ginger (or red ginger). This study uses ginger emprit because it is easy to obtain, affordable, and there has been no research on the content of ginger emprit compounds. Generally, all three types contain starch, essential oils, fiber, a small amount of protein, vitamins, minerals, and a proteolytic enzyme called zingibain[3] Beyond its use as a cooking ingredient, ginger has been empirically used as a component in various medicinal concoctions: such as remedies to boost immunity, combat inflammation, treat coughs, heal wounds, and counteract insect bite allergies.[4]

As a traditional medicinal plant, ginger is utilized to alleviate symptoms related to the throat and tongue, eliminate heart disturbances, and treat vomiting, ascites, cough, dyspnea, anorexia, fever, anemia, flatulence, colic, constipation, swelling, elephantiasis, and dysuria. Additionally, ginger has been used in treating diarrhea, cholera, dyspepsia, neurological diseases, diabetes, eye conditions, and ear inflammation.[5] The composition of ginger has been proven to have antibacterial effects. Research has identified terpenes as the key compounds responsible for these antibacterial properties. Against several microorganisms, terpenes act as bacteriostatic agents. These compounds can interact with the bacterial cell membrane, disrupting its permeability and subsequently impeding the transport of ions in and out of the cell. This disturbance in ion transport can disrupt the proton motive force, which in turn interferes with the energy production process within the cell.[6]

Secondary metabolites are chemical compounds produced by plants in small amounts and have no direct influence on plant growth and development.[7] Metabolite products produced by plants have specific properties and different levels for each species and plant part. In addition, differences in the content of metabolites produced can also be influenced by environmental geographical conditions such as altitude, temperature, rainfall, and soil type.[8] In this study, samples were taken from 14 different growing places in addition to geographical conditions; emprit ginger

rhizomes are cultivated directly by farmers not from wild plants so that the harvest age is uniform.

In addition to serving as a key ingredient in the manufacture of traditional and modern medicines, the antioxidants and antibacterial secondary metabolites produced by *Zingiberaceae* plants can generally inhibit the growth of harmful human pathogens[8] These include *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and fungi such as *Neurospora, Rhizopus, Penicillium, Candida albicans*, and *Microsporum gypsiium*, all of which can cause mycotic diseases in humans and animals[10]. The microbial test subject used in this study was *Staphylococcus aureus*, a Gram positive, cocci-shaped bacterium that can exist individually, in pairs, or clusters.[10] This organism is found in the nasal cavity and skin and is a dangerous pathogen because it causes several diseases such as skin and respiratory infections. This bacterium can become a pathogen if it has the opportunity to enter the body, such as during the use of medical devices. It is most commonly associated with skin infection diseases. Infected body tissues will cause inflammation, necrosis, and abscess formation. [9] In addition to *Staphylococcus aureus*, this study also used the test microbe *Escherichia coli*, a Gram negative bacterium normally present in the gastrointestinal tract of humans and animals. This bacterium can also cause urinary tract infections and diarrhea.[11]

The fresh extract of ginger rhizome demonstrates the capacity to inhibit the growth of test microbes, as observed by the average diameter of the resultant microbe-free zones [12] This phenomenon is attributable to the antimicrobial compounds found in the fresh extract of ginger rhizome, which contain several essential oil components.[13] The ginger rhizome extract exhibited the largest inhibitory zone diameters against two test microbes, specifically, 15.83 mm for *Staphylococcus aureus* and 15.33 mm for *Escherichia coli*.[14] According to the inhibition capacity categorization, the ginger rhizome extract has a moderate inhibitory effect on the growth of *Staphylococcus aureus* and *Escherichia coli*.[15]

In addition to serving as a key ingredient in the manufacture of traditional and modern medicines, the antioxidants and antibacterial secondary metabolites produced by *Zingiberaceae* plants can generally inhibit the growth of harmful human pathogens [16]

MATERIAL AND METHOD

Material

The materials utilized in this study include emprit ginger rhizomes sourced from various cultivation lands in Ponorogo, Magetan, Pacitan, Wonogiri, Karanganyar, Boyolali, Semarang, Magelang, Purworejo, Temanggung, Wonosobo, Banyumas, Bantul, and Kulonprogo, Luria Bertani media, anhydrous Na_2SO_4 (Emsure, Germany), DMSO (Sigma Aldrich, USA), n-hexane (Emsure, Germany), agar (Himedia), distilled water, (Pyrex) 70% alcohol (Smartlab), *Staphylococcus aureus* bacteria (ATCC 25923), ampicillin (Sigma Aldrich) (as a comparative raw material), disposable 96 well microplates, disposable petri dishes, white tips, yellow tips, blue tips The instruments employed in this research include *Gas Chromatography Mass Spectrometer* (GCMS) (Shimadzu QP-2010S), micropipettes, distiller, analytical balance, autoclave, incubator, Laminar Air Flow (LAF) cabinet, (Airegard Work Station) vortex mixer, spectrophotometer UV (Optima SP-3000 nano), and microplate reader (Spark Tecan).

Method

Bacterial culture

Preparation of solid media as a place for bacterial culture was made using Luria Bertani media: agar with a ratio of 2.5 : 1 gram dissolved in 100 mL distilled water. Solid media was placed in Petri dishes for bacterial growth. Pure culture of Gram-positive *Staphylococcus aureus* bacteria (ATCC 25923) was taken 1 dose from glycerol stock and then inoculated into Luria Bertani agar media aseptically, and incubated for 1x24 hours at 37ºC in an incubator.[17]

Antibacterial activity by microdilution method

Antibacterial activity test on essential oil of emprit ginger rhizome was conducted by microdilution method. Bacterial culture on Luria Bertani agar media was suspended in 10 ml of Luria Bertani liquid media aseptically, then incubated in an orbital incubator for approximately 2.5 hours with the aim of obtaining the growth phase of bacteria in the log phase until an optical density (OD) value of 0.25-0.30 was obtained using a spectrophotometer at a wavelength of 600 nm.[18] The bacterial suspension obtained was then used for antibacterial activity testing, no more than 30 minutes after measuring the absorbance of the bacterial suspension.[19]

One essential oil sample was made into three series of levels in the antibacterial activity test. A total of 40 µL of 100% DMSO was placed in a microtube, added 40 µL of essential oil, then added with sterile distilled water up to 1 mL and homogenized. The stock solution had an essential oil concentration of 4% v/v. A total of 800 µL, 700 µL, 600 µL, 500 µL, and 400 µL of the stock solution were placed into five microtubes, then sterile distilled water was added to 1 mL of each, so that the concentrations were 3.2%; 2.8%; 2.6%; 2.0% and 1.6% v/v, respectively. The essential oil concentration series solution was used in the microdilution method antibacterial activity test. A total of 25 µL of essential oil concentration series was placed in the wells of 96 wells microplate added with 150 µL Luria Bertani liquid media and 25 µL bacterial suspension, so that the final concentration of essential oil was 0.4%; 0.35%; 0.3%; 0.25% and 0.2% v/v. The antibacterial activity test was conducted on 96 wells microplate. The wells were divided into treatment sample wells with different concentrations, positive control wells, negative control wells, and blank wells.[20] Each of the treatments, positive control, negative control, and blank wells, was repeated three times to verify the accuracy of the results. Each side of the lid of the 96 wells microplate was glued with parafilm and incubated at 37ºC for 16-18 hours, then the optical density in each well was viewed with a wavelength of 600 nm. [21] The absorbance was read using a microplate reader at a wavelength of 600 nm, then the % inhibition of each concentration was obtained. The percent inhibition was analyzed using probit; from the % inhibition and probit analysis, the MIC_{50} result were obtained.

RESULTS AND DISCUSSION

The Essential Oil Yield

The steam distillation yield of essential oil from the rhizomes of emprit ginger sourced from different regions was as follows: Ponorogo, 0.175% v/b; Magetan, 0.242% v/b; Pacitan, 0.200% v/b; Wonogiri, 0.300% v/b; Karanganyar, 0.213% v/b; Boyolali, 0.183% v/b; Magelang, 0.173% v/b; Semarang, 0.253% v/b; Purworejo, 0.133% v/b; Temanggung, 0.167% v/b; Wonosobo, 0.286% v/b; Banyumas, 0.270% v/b; Bantul, 0.187% v/b; and Kulonprogo,

Table 1. Compounds with Highest Percentage Areas.(Basic data, 2022)

Compounds	Regions	Formula	Highest areas (%)
Camphene	Semarang	$C_{10}H1_6$	10.315
1,8-Cineole	Temanggung	$C_{10}H_{18}0$	8.16
Linalool	Pacitan	$C_{10}H_{18}0$	2.845
3-Cyclohexene-1-methanol, ,alpha,,,alpha,,4-trimethyl-, (S)- (CAS) p-Menth-1-en-8-ol, (S)-(-)-	Semarang	$C_{10}H_{18}0$	1.89
Z-Citral	Wonogiri	$C_{10}H_{16}0$	21.38
Citral	Pacitan	$C_{10}H_{16}0$	26.03
Geranyl acetate	Purworejo	$C_{12}H_{20}O_2$	7.715
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS) ar-Curcumene	Boyolali	$C_{15}H_{22}$	4.31
Zingiberene (CAS)	Karanganyar	$C1_5H_{24}$	5.59
Farnesene	Ponorogo	$C_{15}H_{24}$	4.205
Beta,-Sesquiphellandrene (CAS)	Magelang	$C_{15}H_{24}$	4.19
3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-, [3R-(3,alpha,,3a,alpha,,8a,alpha,)]- (CAS)	Semarang	$C_{15}H_{26}0$	2.28

0.036% v/b. The essential oil content of the emprit ginger rhizome was not less than 0.80% v/b.[22] The distillation yield of essential oil from the emprit ginger rhizomes across these 14 locations was lower than the standard set by FHI. In this study, the rhizomes intended for distillation were dried in an open-air environment for a significantly longer duration than the time taken for harvesting. This process allowed for the evaporation of the essential oil. The difference in essential oil yields could be attributed to the post-harvest treatment of the rhizomes, such as the drying process and slicing, which could reduce essential oil yield.

Identification of Essential Oil Compound Groups from Ginger in 14 Regions via GCMS

The chromatogram profiles of the constituent compounds of the essential oil were analyzed in duplicate with *Gas Chromatography Mass Spectrometry* (GCMS). GCMS analysis showed that the 14 regions respectively had between 48 and 50 compounds. The peak area produced will correlate with the detected compounds; hence, the larger the peak area, the greater the number of secondary metabolites. The 14 essential oil growth locations in ginger rhizomes had similar numbers of compounds, but there were differences in compound concentration presentation in each sample. The essential oil components were chosen for identification based on peak criteria with an area greater than 0.5%. Emprit ginger rhizomes grown in Pacitan contained 12 monoterpene group essential oil components, with Citral having the highest area percentage of 26.03%. The details can be seen in Table 1.

Antibacterial Activity

Antibacterial activity was investigated by optimizing DMSO on *Staphylococcus aureus* bacteria. It was observed that DMSO at a final concentration under 0.8% v/v did not inhibit the growth of either bacteria. The antibacterial activity of essential oil from ginger rhizomes collected from 14 different growth regions was tested using DMSO at a final concentration of less than 0.5% v/v.

Additionally, *Staphylococcus aureus* bacteria contain teichoic acid, a water-soluble polymer, suggesting that the cell wall is polar. The non-polar nature of the ginger rhizome essential oil sample makes it more challenging to penetrate the polar cell wall of *Staphylococcus aureus* bacteria. The essential oil from ginger rhizomes contains oils derived from the terpenoid group. The mechanism of

terpenoids in antibacterial activity occurs at the transmembrane proteins located on the outer membrane of the bacterial cell wall. The essential oil can form strong polymer bonds until damage occurs to the transmembrane proteins, which serve as pathways for compound exchange. This damage results in a nutritional deficiency in bacterial cells that may inhibit bacterial growth, leading to eventual cell death.

Principal Component Analysis

The Principal Component Analysis (PCA) objective in the similarity analysis of essential oils from emprit ginger rhizomes from 14 different growth locations is to group correlated variables and replace them with a new group known as principal components. PCA is conducted to comprehend the relationship between the distribution of essential oil compound contents and the growth locations of the emprit ginger rhizome samples. The results of the PCA score scatter plot analysis are depicted in Figure 1.

The PCA results in a *score plot* curve can be utilized to estimate the data structure, serving as a basis for differentiating essential oils from emprit ginger rhizomes based on geographical disparities. The distance between samples indicates the similarity between the samples. Far and near distances among samples exhibit the extent of similarity among them. Essential oils from emprit ginger rhizomes from 14 growth locations lie in distinct quadrants. It suggests the presence of differences in the characteristics of essential oil compound contents of emprit ginger rhizomes from these 14 different growth locations. Essential oils from emprit ginger rhizomes with growth locations in Ambarawa (Semarang), Wonosobo, Temanggung, Karanganyar, Kulonprogo, and Magetan have similar percentage areas, possibly indicating similar compound contents.

However, areas with larger percentage differences exhibit differing compound contents. The areas of Kulonprogo and Magetan show the same trend. The processed results of the PCA of essential oils from emprit ginger rhizomes in the form of a *score plot* are presented in Figure 1.

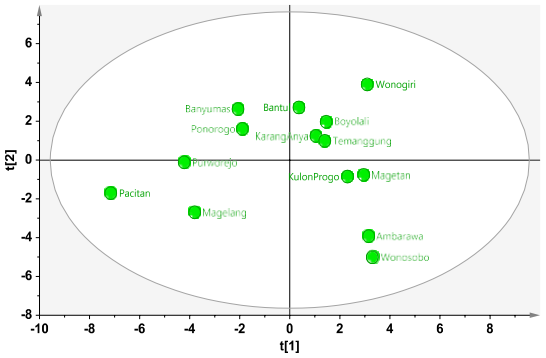

Figure 1. PCA *score plot.*

In thehe OPLS DA analysis, Figure 2 shows the results for compounds influencing the growth of emprit ginger across 14 locations are presented. Compounds that significantly impact the growth of emprit ginger across these 14 locations include *Citral, (2R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, Z-Citral, Geranyl acetat, Camphene, (6E)-octa-1,6-diene, 7-methyl-3-methylideneoct-6-enal, Farnesene, Bornylene, Beta-Myrcene, Zingiberene, Alpha-pinene*.

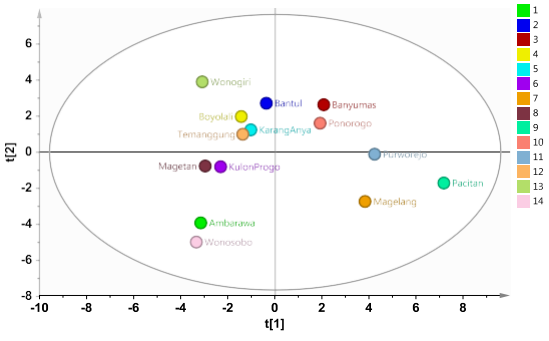

Figure 2. OPLS DA for Discriminating Compounds Inside Emprit Ginger.

The compounds influencing the MIC_{50} against *Staphylococcus aureus* bacteria with GCMS variables include *Z-Citral; Geranyl asetat; Zingiberenol; Beta-Myrcene; (1S)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene; and (2R)-1,7,7-trimethylbicyclo [2.2.1] heptan-2-ol.*
The graphical results are presented in Figure 3:

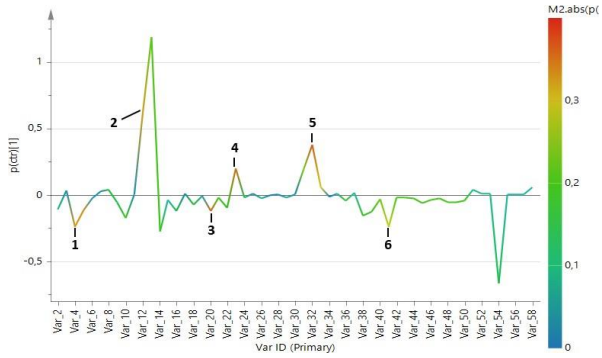

Figure 3. OPLS of *Staphylococcus aureus* Bacteria with GCMS Variables.

Correlation of Essential Oil Profiles from Ginger with Growth Location and its Antibacterial Activity

The antibacterial activity of *Staphylococcus aureus* on essential oils from the rhizomes of fingerroot ginger, grown at varying locations was discerned. The results of the antibacterial activity test of *Staphylococcus aureus* on the essential oil of emprit ginger rhizome from Boyolali area has the highest MIC_{50} value of 0.2011% v/v.

STRENGTH AND LIMITATION

The study on emprit ginger rhizomes presents a commendable breadth of analysis, encompassing essential oil yield data sourced from 14 distinct regions, complemented by the rigorous analytical technique of *Gas Chromatography Mass Spectrometry* (GCMS) applied in duplicate. This methodological depth is further enriched by the exploration of the antibacterial activity of the oils, particularly against *Staphylococcus aureus*, thus offering a functional perspective alongside compositional insights. Additionally, the employment of *Principal Component Analysis* (PCA) sheds light on the intricate relationships between different samples based on their essential oil profiles, with the identification of key compounds serving as a pivotal contribution to understanding the growth and antibacterial properties of emprit ginger. However, while the research is thorough, it is not without limitations. The open-air drying method employed for the rhizomes before distillation may have compromised the oil yield, introducing potential bias. This is coupled with a detectable variability in the compounds observed between the two GCMS replications. Moreover, the singular focus on *Staphylococcus aureus* limits the study's antibacterial scope, and the absence of comparative MIC_{50} values to a reference makes discerning the potency challenging. Furthermore, the lack of detailed results from the PCA and potential absence of controls in antibacterial testing call for cautious interpretation. Lastly, while the study provides valuable regional insights, external factors such as soil quality, climatic conditions, and specific farming practices were not considered, possibly impacting the essential oil yield and composition. Overall, the study stands as a significant contribution to the domain, but considerations regarding its limitations are crucial for a holistic understanding.

CONCLUSIONS

The essential oil from the ginger rhizome, derived from 14 different geographic locations, demonstrates a significant variation in MIC_{50} values against *Staphylococcus aureus* bacteria. The results of the antibacterial activity test

of *S. aureus* on ginger emprit rhizome essential oil derived from Boyolali area has the highest MIC_{50} value of 0.2011% v/v. Analysis of antibacterial activity of compounds that showed significant discriminatory effects on *Staphylococcus aureus* bacteria are *Z-Citral; Geranyl asetat; Zingiberenol; Beta-Myrcene; (1S)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene; and (2R)-1,7,7-trimethylbicyclo [2.2.1] heptan-2-ol*. These have shown impactful effects on the *Staphylococcus aureus* bacteria.

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CONFLICT OF INTEREST

All authors have no conflict of interest

AUTHOR CONTRIBUTION

AAS: Conceptualization, Methodology, Software, Resources, Data Curation, and Writing Original Draft; **P**: Conceptualization, Methodology, Formal analysis, Writing, Review and Editing, and Supervision; RA**S**: Writing, Review and Editing, Visualization, and Supervision; **AW**: Writing, Review and Visualization; **AR**: Writing, Review and Supervision.

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Original Article

IJTID

(INDONESIAN JOURNAL OF TROPICAL AND INFECTIOUS DISEASE)

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Effect of Immunization of the Pili Protein 65.5 kDa *Klebsiella pneumoniae* on IFN-γ Levels of Spleen BALB/c Mice

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Abstract

Klebsiella pneumoniae is a Gram-negative bacterium that poses a threat to the global community. Currently, no vaccine for *K. pneumoniae* is licensed by the Food and Drug Administration (FDA). The delay in the manufacture of the *K. pneumoniae* vaccine was because many vaccine candidates failed at the clinical trial stage due to adverse cross-reactions. Pili can be used as a choice as a vaccine candidate. Pili *K. pneumoniae* is an immunogenic substance that triggers an immune response, one of which is the cytokine IFN-γ. Splenic splenocytes are the main source of IFN-γ-producing cells. The aim of this study is to determine the effect of immunization pili protein 65.5 kDa *K. pneumoniae* on IFN-γ levels from spleen BALB/c mice. There were 3 groups, K1 as control given PBS, K2 given pili protein 65.5 kDa + adjuvant, and K3 given adjuvant. IFN-γ was then measured by the ELISA method and analyzed by the ANOVA test. The results of measuring IFN-γ levels using one-way ANOVA showed that the total for all groups was 243.50 ± 43.7 with $p < 0.05$, the Post Hoc LSD test was continued. The Post Hoc test showed significant differences between K1 control and K2 groups, and between K1 and K3 groups, but not between K2 and K3 groups. It can be concluded that immunization with 65.5 kDa of pili protein does not affect the increase in IFN-γ levels in the spleen of BALB/c mice.

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INTRODUCTION

Klebsiella pneumoniae is a Gram-negative bacterium that is a threat to the global community. The rate of resistance to ciprofloxacin, a commonly used antibiotic, increased from 4.1% to 79.4%.[1] Increased isolation multi-drug resistant (MDR) *K. pneumoniae* narrowed the options for K. *pneumoniae* infections treatment.[2] According to data from Perhimpunan Dokter Paru Indonesia (PDPI), the most frequent cause of community pneumonia was *K. pneumoniae* (29%), followed by *Staphylococcus aureus* (16%) and *Streptococcus pneumoniae* (12%).[3] Riset Kesehatan Dasar (Riskesdas)[4] shows an increase in the prevalence of pneumonia caused by *K. pneumoniae* in Indonesia, increasing from 4.5% in 2013 to 5.0% in 2018. East Java has a high prevalence of 4.4%.

Currently, there is no vaccine approved by the FDA (Food and Drug Administration) against *K. pneumoniae* infections. The delay in the production of the *K. pneumoniae* vaccine was because many vaccine candidates failed at the clinical trial stage due to adverse cross-reactions. However, pili virulence factors have low antigenic variation and low cross-reactivity, making them suitable as a vaccine candidate.[5,6] Pili play a role in the attachment of bacteria to the surface of the human mucosa or epithelium. The advantage of pili is that the structure easily triggers the formation of antibodies compared to other virulence factors.[5,7]

Pili proteins *K. pneumoniae* from previous studies were known to have varying molecular weights. In research, Agustina et al. (2021)[8] tested the IgG response with Western Blot (WB) showing that the proteins that appeared were 85.6, 65.5, 46.9, and 29.4 kDa. Pili protein 65.5 kDa is band thickest. Pili *K. pneumoniae* is immunogenic and triggers immune responses, such as the production of cytokines, one of which is IFN-γ which is produced by T-helper (Th1) cells, natural killer (NK) cells, NKT, and CD8+ T cells.[9] Significant IFN-γ production occurs in the lungs, liver, and spleen after infection with *K. pneumoniae*. Splenic splenocytes are the main source of IFN-γ-producing cells.[10] IFN-γ will activate macrophages and B lymphocyte cells. B lymphocyte cells will stimulate the production of immunoglobulin G (IgG), which can prevent infection with *K. pneumoniae*.[11] Based on the above background, researchers are interested in testing the ability of the 65.5 kDa *K. pneumoniae* in inducing IFN-γ cytokines in the spleen of mice.

MATERIALS AND METHODS

Identification of *Klebsiella pneumoniae* Pili Protein Molecular Weight

The Laemmli research method is used to identify the pili protein weight using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).[12] The gel components used are separating 12.5% and stacking 4%. Samples were run for 10 wells with nine wells containing samples and one well-containing protein marker of 45 µl, and buffer samples in the same volume. The color used was bromophenol blue. After the sample was added sample buffer, it was heated to 95°C for four minutes, and then it was ready to be inserted into each well after the gel was submerged in buffer. The voltage used was 120 mV, the current 400 mA, and run for 60 minutes. SDS-PAGE results were determined using a linear equation according to protein markers using MS Excel curve fitting so that the molecular weight of the pili protein can be read.

***Klebsiella pneumoniae* Pili Protein Purification**

Weight of the protein that forms the band resulting from electrophoresis was cut according to the required weight. The results of the cut bands sheet nitrocellulose were then mixed with sterile PBS for electroelution in an electroelution chamber filled with buffer. The nitrocellulose sheet was clamped tightly on both sides until the two sides were tight. The power supply was set to 125 mV, 0.3 A for 120 minutes. A beaker glass containing 1 liter of sterile PBS with a pH of 7.4 was prepared for dialysis of the electroeluted sample. The magnetic stirrer was inserted into the glass beaker, ensuring that the magnetic stirrer continued to rotate after being put in refrigerator for 2 x 24 hours. Every 24 hours sterile PBS was replaced with new sterile PBS. Dialyzed target protein samples were taken and stored in a refrigerator at -85°C. Protein content of purified proteins was determined by the Kingsley method.[8]

Mice Acclimatization

Acclimatization was carried out by keeping mice for seven days at the Experimental Animal Laboratory, Faculty of Medicine, Jember University. After seven days, the mice were randomized before being immunized according to the treatment group.

Immunization of Mice with Pili Protein 65.5 kDa

Immunization of white male mice with BALB/c strain aged 6-8 weeks was administered intraperitoneally three times with an interval of 14 days. There were three treatment groups, K1 (control) was given 0.2 ml of sterile PBS, K2 was given 50 µg of pili protein and Freund's adjuvant with the same volume as the diluted antigen volume, which was 0.1 ml, and K3 was given of Freund's adjuvant and 0.1 ml of PBS. In priming, Complete Freund's Adjuvant (CFA) was used, while Incomplete Freund's Adjuvant was used for booster.[13,14]

Termination of Mice

Fourteen days after the third immunization, the mice were terminated with cotton soaked in ether. An incision was made in the abdomen of the mice, then the spleen was separated and taken. Spleen organs were taken, washed with PBS pH 7.4, and then weighed. After that, the organs were chopped using a mortar and stamper on ice and then homogenized with PBS. In the final step, the organ was centrifuged at 2000-3000 RPM for 20 minutes.

Measurement of IFN-γ using the ELISA Method

Measurement of IFN-γ method Sandwich ELISA kit Bioassay Technology Laboratory)® No. E0056Mo from Shanghai Korain Biotech Co Ltd, China.

Statistical analysis

A one-way ANOVA test was used to assess IFN-γ in each group in order to identify the differences in each sample group. Final data were processed using IBM SPSS Statistics 24.

RESULTS AND DISCUSSION

Identification and Isolation of Pili Protein 65.5 kDa *K. pneumoniae*

K. pneumoniae's SDS-PAGE results revealed that there was a band with a molecular weight of 65.5 kDa. The band with a molecular weight of 65.5 kDa was thicker, so this band was isolated to be immunized in experimental animals. The results of protein purification were then measured to see the concentration and the protein yield reached 0.65 g/dl.

The protein identification results can be seen in Figure 1.

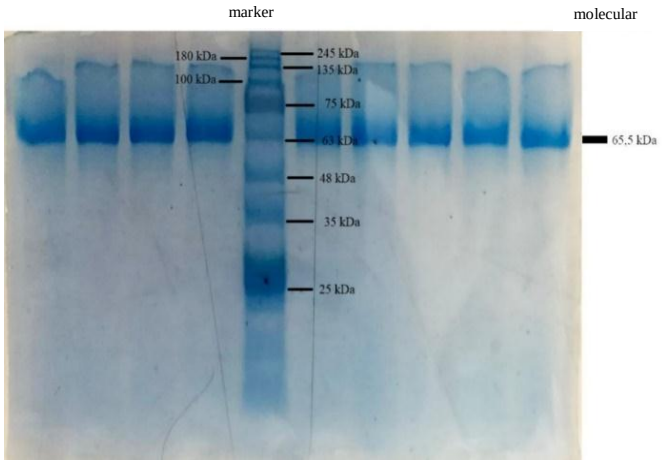

Figure 1. The results of SDS-PAGE protein pili *K. pneumoniae.* The band with a molecular weight of 65.5 kDa is thicker.

IFN-γ

The results of measuring IFN-γ cytokine levels using ELISA showed the highest average IFN-γ levels in the adjuvant group, followed by the adjuvant+antigen group, and the control group showed the lowest average IFN-γ levels. The results of the measurement of IFN-γ cytokine levels are presented in Table 1. The normality test was used in the study using the Shapiro-Wilk test. In the test results, it was found that the data had a normal distribution with all groups having a significance value of $p > 0.05$.

Table 1. Results of Measuring Levels of IFN-γ

	N	IFN-γ (*Mean* ± *SD*)
K1 (Control)	7	208.16 ± 24.69
K2 (Antigen + Adjuvant)	7	252.07 ± 32.36
K3 (Adjuvant)	7	270.27 ± 48.29
Total	21	243.50 ± 43.71

Results of Data Analysis

One-way ANOVA was used to assess IFN-γ levels, as shown in Table 2. The one-way ANOVA analysis of variance yielded $p < 0.05$ as its results. Between the groups in this study, there were notable variances or significantly different.

Table 2. Comparative Test Results One-way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	14272.708	2	7136.354	5.367	.015
Within Groups	23932.410	18	1329.578		
Total	38205.118	20			

One-way ANOVA revealed significant differences between the group means, and Post Hoc LSD tests were continued as shown in Table 3. Post Hoc LSD tests revealed significant differences between control and antigen+adjuvant groups ($p = 0.037$), and between control and adjuvant groups ($p = 0.005$), but not between the antigen+adjuvant group and adjuvant group ($p = 0.363$). The significant difference can be seen from the asterisk (*) behind the number in the mean difference.

IFN-γ levels were significantly different ($p < 0.05$) between the groups, according to the results of the statistical test using one-way ANOVA. This shows that the administration of antigen+adjuvant or adjuvant treatment alone can increase IFN-γ levels. Similar to the study of Ramsugit et al. (2016)[15] using pili protein *Mycobacterium tuberculosis*, IFN-γ levels differed significantly with a one-way ANOVA showing $p<0.05$. Post Hoc LSD analysis showed that IFN-γ levels in control and antigen + adjuvant groups were significantly different ($p < 0.05$). This was

Table 3. Post Hoc LSD Test Results

Groups	Groups	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Adjuvant + Antigen	-43.911565*	19.490498	.037	-84.85958	-2.96355
	Adjuvant	-62.108844*	19.490498	.005	-103.05686	-21.16083
Adjuvant + Antigen	Control	43.911565*	19.490498	.037	2.96355	84.85958
	Adjuvant	-18.197279	19.490498	.363	-59.14530	22.75074
Adjuvant	Control	62.108844*	19.490498	.005	21.16083	103.05686
	Adjuvant + Antigen	18.197279	19.490498	.363	-22.75074	59.14530

* The mean difference is significant at the 0.05 level.

in line with the research of Udin et al. (2014)[16] which showed significantly higher IFN-γ secretion in the group injected with *M. tuberculosis* protein compared to the control group with $p < 0.05$. According to the theory, pili protein can trigger immune responses, one of which is IFN-γ. When the host is injected with *K. pneumoniae* pili protein, the host cell will elicit an immune response in the form of T-helper. T lymphocytes will activate inflammatory cytokines, one of which is IFN-γ.[7,17] When pili antigen is combined with Freund's adjuvant, it will stimulate the immune system more strongly. These adjuvants contain immunoreactive molecules that induce IFN-γ.[17,18] This indicates that in this study there was an effect of the antigen+adjuvant administration on increasing IFN-γ levels compared to PBS alone in the control group.

IFN-γ levels between control and adjuvant groups were significantly different ($p < 0.05$). These results follow a study conducted by Udin et al. (2014)[16] which the adjuvant-only injected group had significantly higher IFN-γ secretion in the group than the control group, $p = 0.000$ ($p < 0.05$). The increase in IFN-γ could be due to the choice of adjuvant used in this study. Freund's adjuvant consists of N-acetylmuramil-l-alanyl-d-isoglutamine (muramyl dipeptide) and trehalose 6,6'-dimycolate, which are immunoreactive molecules that can induce IFN-γ thereby polarizing T-helper (Th1) cells.[19] Freund's adjuvant has several disadvantages, namely that it can cause granulomas, inflammation, and lesions.[20] Lesions may lead to inflammation, and it can boost the release of cytokines, such as IFN-γ.[21] This shows that the adjuvant group can affect increasing levels of IFN-γ compared to the control group.

IFN-γ levels between the antigen+adjuvant group and the adjuvant group showed no significant difference ($p > 0.05$). This shows that pili protein immunization does not affect increasing IFN-γ levels in the spleen. These results follow a study conducted by Ayu.[22] The results showed that even in livers injected with pili 65.5 kDa, IFN-γ levels did not significantly differ between the adjuvant and antigen+adjuvant groups, $p = 0.511$ ($p > 0.05$). *K. pneumoniae.* The absence of a significant difference in IFN-γ levels could be due to the sample being measured in this study being tissue, not blood circulation. IFN-γ is produced by T lymphocytes (Th1) cells produced by lymphoid organs and then flows through the blood circulation and empties into the organs that have dendritic cells, one of which is the spleen. Then it will migrate into the lymphatic tissue, then migrate back into the blood circulation.[11,23] IFN-γ which will migrate back to the blood circulation causes IFN-γ levels to be more

measured in the blood circulation. This is consistent with the findings of Martínez-Orellana et al. (2022)[24], who showed that the concentration of IFN-γ measured in blood serum increased after immunization of the Leishmania infant. This shows that the increase in IFN-γ levels when given *K. pneumoniae* will be more or significantly measurable in the circulation than in the tissue.

The absence of significant differences in IFN-γ levels between the antigen+adjuvant group and the adjuvant group could also be due to the type of adjuvant used in this study. Freund's adjuvant is an adjuvant based on mineral oil emulsions. Vaccines containing oil emulsion-based adjuvants such as Freund's adjuvant can cause the antigen release process to be slower, but the immune response formed can last longer in the body.[19,25] When the adjuvant is combined with the antigen, it can slow down the release of the antigen to induce IFN-γ, so that the level of IFN-γ measured is lower than the adjuvant group alone. This could be the reason that caused the level of IFN-γ in the antigen+adjuvant group (252.07 pg/ml) to be lower than the adjuvant group (270.27 pg/ml) in this study, due to the length of IFN-γ response formed in the antigen+adjuvant group.

STRENGTH AND LIMITATION

The strength of this study is that this *K. pneumoniae* pili protein is available as a vaccine candidate, which will be useful in the development of future vaccines for *K. pneumoniae* infections. The limitation of this research pertains to there are no groups for only antigen without adjuvant. For future research, it is hoped that there will be only antigen groups without adjuvant in research, so that maximum and unbiased results are obtained.

CONCLUSIONS

From the results of this study, it can be concluded that immunization with 65.5 kDa of pili protein does not affect the increase in IFN-γ levels in the spleen of BALB/c mice.

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ETHICAL CLEARANCE

Ethical approval letter (No. 1565/H25.1.11/KE/2022) was obtained from the Ethics Committee of the Faculty of Medicine of the Jember University for this study.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

Writer, literature searcher, collecting data from literature : AS, concept and supervision : DA, review and supervision: DCM and PWP.

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Scientific Journal of Tropical and Infectious Disease

Early Detection of Infectious Diseases among the Refugees of UNHCR in South Tangerang, Banten; the Problems and Strategies to Prevent the Disease's Transmission

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Abstract

The previous study at Puskesmas Pisangan, Ciputat had reported that 23.8% of patients of the UNHCR was infected by malaria *Plasmodium vivax*, and one patient with bacterial urinary infection. However, the result cannot represent the actual case of the disease, because of the lack number of participant to visit the Puskesmas since the Covid-19 pandemic which had been contributing to decrease number of the patients**.** The study purposed to improve data and information about parasitic infection, and to design strategy in early detection and prevention to the disease. Design of the study was approached in cross-sectional with a total sampling method of the UNHCR out patients visiting the Puskesmas Pisangan and Cirendeu. We collected specimen of feces, urine, and blood, and performed blood diff-count, rapid diagnostic, microscopic, dipstick, and bacterial culture. The study revealed some parasitic and bacterial infections as defined: five cases (17.24%) of malaria, which is suspected as imported cases; *Enterobacteriacea* family as non-specific bacteria of negative gram in urine; also *Entamoeba coli* in stool. This finding was also confirmed 17.24% of leucocytosis in differential blood count and 24.14% in urinalysis. By nationality, Sudanese was detected the most prevalent 10.34% of parasitic infections, followed by Somalian (6.9%), Yemeni (3.45%), and Afghan (3.45%) respectively. Mosquitoes and poor living conditions also contributed as the major potential risk of transmission to the diseases. In conclusion, early detection, health screening, vaccination, access to primary, and upgraded levels of healthcare are important for diseases control and management to prevent the transmission.

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INTRODUCTION

The United Nations High Commissioner for Refugees (UNHCR) in Indonesia has reported about 13,416 persons registered with UNHCR in Indonesia, 73% adult and 27% children. By April – June 2021, UNHCR reported that most of the refugees coming to Indonesia were from Afganistan (55%), Somalia (10%), Myanmar (5%), Sudan (3.8%), Iraq (0.05%), and other countries (19.5%).[1] In June 2021, the study at Puskesmas Pisangan on Ciputat, South Tangerang District reported some cases of parasitic infection in the refugees detained at the study area. Malaria and bacterial urinary infection were evidently detected as the major cases within the refugees.[2]

Several studies have reported that the origin of country of the refugees seemed to correlate with parasite infection burden within the refugees. For example, pathogenic intestinal parasites were found in 40.6% of the 956 African asylum seekers coming from areas south and east of the Sahara, and helminthiasis infections Strongyloidosis and Schistosomiasis were identified in 15% of patients from Africa.[3] Malaria and other intestinal parasites are widely prevalent in developing countries such as Indonesia, due to poor sanitation, inadequate personal hygiene, etc. This disparate case distribution is probably due to a complex combination of factors related to exposure infection, such as environmental and host behavior, and factors related the host's ability to resist infection, such as the genetic constitution and immune responsiveness (cited in Martin and Mak, 2006).[4]

The study purposed to give a broad overview and raise concern about the infectious diseases in the refugee and asylum seeker populations in the present time. Many areas in Indonesia, including South Tangerang District as the area of study, have for many years been resettled for the UNHCR refugees. However, the availability of evidence of demographic data and health status of the refugee and their life condition in this area is little reported.

On the other hand, several reports are published on health problems and access to healthcare for the UNHCR refugees around the world. Many aspects were identified to hamper the problems such as legality status of the refugee, barrier of language and communication, physical disability, poverty, etc. Yet, the available evidence on health problems among asylum seekers and refugees is limited in general with the best documentation on infectious diseases, mental illness, maternity health and almost non-existing for chronic diseases and childhood illnesses.[5]

Poor data and information about health status in the refugee in Indonesia may also be an obstacle as well as needing to initiate a better understanding about the possibility of disease transmission among the refugees and their local community. The risk of transmission to the autochthonous population is very low, though outbreaks in the refugee population should be considered due to poor living conditions and suboptimal vaccination, not least among children. Even though we see high transmission in the refugee populations, there is very little risk of spread to the autochthonous population.[6]

This study explores the potential transmission factors of parasitic diseases among the refugees and their local community. Screening may serve to avoid potential infectious disease risks in the receiving countries as well as to identify health needs of asylum seekers. It may create a two way moral obligation, upon asylum seekers to actively participate in the program, and upon authorities to reciprocate

the asylum seekers' participation and the benefits for the control of public health.[7]

Through this study, we follow up the previous data and extend the area of study in order to find more cases of infectious diseases within the refugees. It is also expected to get more information about some aspects as causative agents and routes of transmission of the diseases. The finding result should be a clear benefit to provide data and information for the local health takers to plan the strategy of health program in early detection, prevention, and treatment to eliminate disease transmission.

MATERIAL AND METHOD

Materials

We utilized some materials to conduct assays and tools for collecting samples. To proceed microbiological procedures, we used media produced by MERCK such as : MacConkey Agar by MERCK KGaA Germany #1.05465.0500 for cultivating Enterobacteriacea colonies; SS Agar by MERCK KgaA Germany #1.07667.0500 for Salmonella-Shigella culture; and Nutrient agar by MERCK KgaA Germany #1.05450.0500. In running the urine test, we used dipstick urinalysis reagent strips by ACON Biotech, Hangzhou, P.R.China. For malaria, we utilized combo rapid test by Zhejiang Orient Gene Biotech Co. LTD, China, which has four indicators of control, Pan malaria, *Plasmodium vivax*, and *Plasmodium falciparum*, then we proceeded malarial stain by giemsa product by MERCK KgaA Germany #1.09261.1000.

Design of the Study

Design of the study was cross-sectional, with total sampling of all UNHCR patients visiting the Puskesmas Pisangan and Cirendeu at Ciputat area. They were priorly examined by clinician for their current and previous historical medical status. All the subjects were required to give agreement to participate in the study by signing the informed consent. They were also required to fulfill a questionnaire in Bahasa, English, and Arabic, which was provided by the researcher. We also collected stool, blood, and urine specimens from the subjects.

Subjects of the study were selected by the inclusion and exclusion criteria of the specimen. The inclusion criteria accommodated the attendance of all the UNHCR/IOM refugees visiting the puskesmas during the study. They were also required to collect specimens of urine, blood, and feces, and complete the questionnaire to attach with the specimen. Those with Covid-19 and/or other suspected infectious diseases who isolated at home-based care, were also considered in this criteria. Meanwhile, those with incomplete questionnaire and specimen were excluded.

Sample Collection

For each patient, we collected blood and urine during their visit to the Puskesmas while the feces were collected at home and returned to the laboratory on the next day. Each specimen was aliquoted to perform different tests and to double check the result. For the urinalysis and routine differential blood count these were documented at the Puskesmas laboratory. while parasitology tests in blood, feces, and urine were performed and analyzed at the laboratory of Parasitology in UIN Syarif Hidayatullah.

Several tests were approached to determine the pathogen in the specimens, such for the intestinal parasite through a stool microscopic; for the blood parasite by utilizing microscopic and rapid test; and for urine samples, we conducted rapid analysis by dipstick reagent strips, and

cultivation procedures to detect bacterial urinary infections in media cultures.

Methods

Malaria Procedure

Laboratory procedure for malaria detection was performed by thick and thin smear. It should be considered before performing smear that blood collected with the use of EDTA anticoagulant is acceptable; however, if the blood remains in the tube for any length of time, true stippling may not be visible within the infected RBCs (*Plasmodium vivax*, as an example). Also, when using anticoagulants, it is important to remember that the proper ratio between blood and anticoagulant is necessary for good organism morphology. Heparin can also be used, but EDTA is preferred. Finger stick blood is recommended, particularly when the volume of blood required is minimal (i.e., when no other hematologic procedures have been ordered). The blood should be free flowing when taken for smear preparation and should not be contaminated with alcohol used to clean the finger prior to the stick.[8]

Thick smears consist of a thick layer of dehemoglobinized (lysed) red blood cells (RBCs). The blood elements (including parasites, if any) are more concentrated (app. 30×) than in an equal area of a thin smear. Thick smears should not be fixed with methanol or heat. If there is a delay in staining smears, the thick smear should be briefly dipped in water to hemolyse the RBCs. Thus, thick smears allow a more efficient detection of parasites (increased sensitivity). However, they do not permit an optimal review of parasite morphology. For example, they are often not adequate for species identification of malaria parasites: if the thick smear is positive for malaria parasites, the thin smear should be used for species identification (Figure 1).[9]

Thin smears consist of blood spread in a layer such that the thickness decreases progressively toward the feathered edge. The smears are fixed by dipping them in absolute methanol and drying in open air. The smears allows an adequate identification of malaria species (higher specificity).[9]

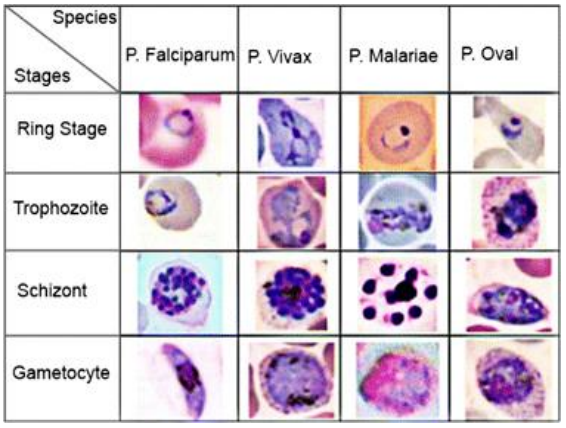

Figure 1. Morphology stages of Plasmodium species.[8,9]

Dipstick Principle, Procedure and Interpretation

The principle of dipstick urinalysis is to detect substances or cellular material in the urine associated with metabolic disorders, renal dysfunction or urinary tract infections (UTI). A totally negative dipstick test is associated with negative microscopy in 90-95% of cases (false negative rate 5-10%). Several indicators of urinary function are measured by color changes on the dipstick chart. The color changes determine the titer of components in urine such nitrate, leucocyte, protein, glucose, bilirubin, hemoglobin, ketone, and pH.[10,11]

The procedure requires diluting the dipstick for three seconds and ensuring that the urine spreads all over it. The color changes on the dipstick urinalysis chart at different time indicators are then interpreted, as shown on the chart (Figure 2).

Bacterial Cultures, Principle, Procedure and Interpretation

We continued to cultivate the urine samples by utilizing three media of cultures by MacConkey Agar (MCA), Nutrient agar

(NA), and *Salmonella-Shigella* agar (SSA). If one of the cultures showed negative result, then we continued to run the other two cultures to isolate *Enterobacteriaceae* colonies.

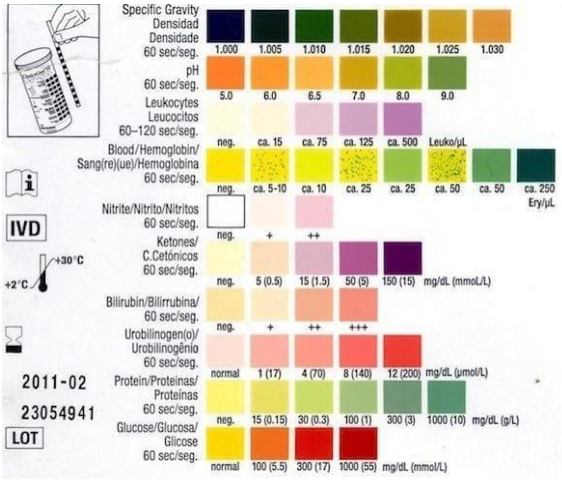

Figure 2. Dipstick urinalysis chart[11]

The principle of MCA is used for the isolation of Gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. MCA is particularly recommended for the cultivation of pathogens which may be present in a variety of specimens such as urine, feces and wound swabs.[12]

We conducted the procedure of MCA as follows: Preparation of MacConkey Agar: 1. Measure 10.3 grams of MCA (MacConkey agar oxoid 51.5 gr/1 L); 2. Mix with distilled water to make 200 ml; 3. Heat the mixture on a hot plate stirrer until cooked; 4. Transfer the mixture to an Erlenmeyer flask and seal it tightly; 5. Pour about 20 ml into a petri dish near a Bunsen flame in a laminar airflow; 6. Store the prepared media in a cool, dry place, such as a refrigerator.

Cultivate bacteria on MCA Media for Urine Culture: 1. Prepare MacConkey agar media in a petri dish; 2. Dip the flared dose into urine then stroke it into MacConkey Agar media; 3. Incubate the media in an incubator at 37ºC for 24 hours, and observe for any microbial growth.

Result interpretation on MCA is indicated positive by red or pink and may be surrounded by a zone of acid precipitated bile for lactose fermenting strains growth such as *Escherichia coli* (Figure 3).[12]

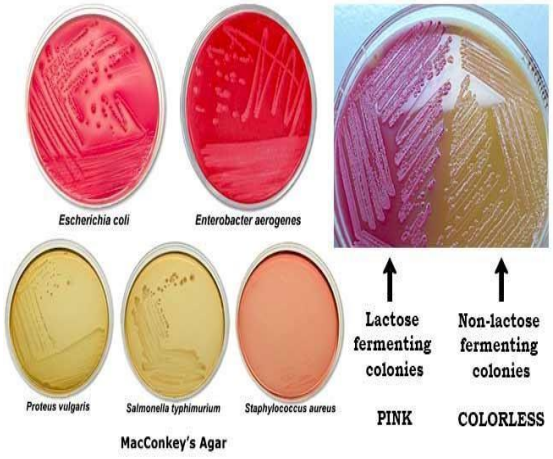

Figure 3. Bacterial colonies on MacConkey agar.[12,13]

We continued to select the bacterial colonies to cultivate in the Nutrient agar (NA). Preparation of NA: 1.Weigh 4 grams of Merck NA media designed for 1 liter of solution; 2. Suspend the weighed media in distilled water, and make the final volume up to 200 ml; 3. Heat this suspension on a hot plate stirrer until it is fully cooked; 4. Transfer the cooked solution into an Erlenmeyer flask and tighten the lid; 5. Pour approximately 20 ml of solution onto a petri dish near a Bunsen flame in laminar airflow; 6. Store in a cool and dry place (in the refrigerator).

Cultivation of Microorganisms from Urine in NA: 1. Prepare a petri dish of Nutrient agar media; 2.Dip an incandescent loop into the urine sample and then streak it onto the NA at 37°C for 24 hours; 4.Observe the media for any signs of microorganism growth.

To confirm selected growth colony on NA, we continued the procedure on *Salmonella-Shigella* Agar (SSA) as follows. Preparation of SSA: 1. Weigh 12 gram of SSA media (SSA Merck 60 gr/ 1 L); 2. Add distilled water

to the media and make the final volume 200 ml; 3. Heat the mixture on a hot plate stirrer until it becomes cooked; 4. Transfer the mixture to an Erlenmeyer flask and close it tightly; 5. Pour about 20 ml of the mixture into a petri dish near a Bunsen flame in laminar airflow; 6. Store the petri dishes in a cool and dry place (in the refrigerator).

Cultivation of Microorganisms from Urine in SSA: 1. Prepare the SSA media by pouring it into a petri dish; 2. Dip a flared tube into the urine sample and then gently stroke it onto the surface of the agar media; 3. Place the petri dish in an incubator and incubate it at 37°C for 24 hours; 4. After 24 hours, observe the petri dish to check for the growth of microorganisms. The results are shown in Figure 4.

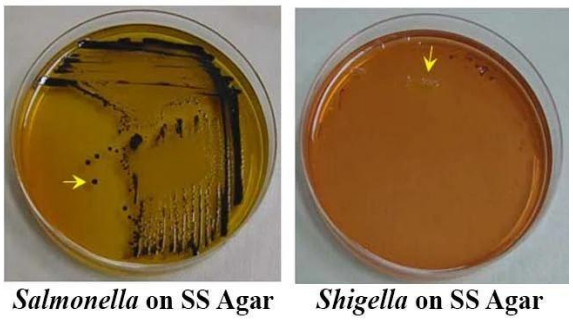

Figure 4. Different color of colonies *growth* on SSA[12,13]

These three types of media should be proceeded to the next confirmative test by Triple sugar iron agar (TSIA) as a selective and differential medium to differentiate bacteria, especially of the *Enterobacteriaceae* family. The TSIA test is a biochemical test used to differentiate bacteria based on their ability to ferment these three sugars and release acid and hydrogen sulfide gas.[13]

We did not conduct the TSIA test since the samples no longer existed and no media was available when we had the colonies on SSA and NA media.

Data Management and Analysis

Data management and analysis were considered to quantitive description for subject's characteristic, parasitic infection, health record, and transmission-related factors. Comparative studies were analyzed by means to find infectious samples of parasite by microscopic, rapid test result, dipstick, and bacterial culture.

RESULTS AND DISCUSSION

We interviewed and collected samples from 50 participants of the UNHCR patients visiting the Puskesmas Pisangan and Cirendeu in Ciputat sub-district. The finding described in the following tables.

Characteristic of the subjects

Several aspects were included to describe the characteristics of subject in the study. We also combined data from the previous and recent study as follows Table 1.

By Table 1, we found that most prevalent of our subjects were men aged between 18-55 years old, and by the nationality was 26% from Somalia. It was also reported by medical records that the most frequent diseases were diarrhea, helminthiasis, and hepatitis. We cannot define and trace the medical history of the diseases as there is no evidence base of infectious diseases in refugees to trace from medical records. This may also be an obstacle to a better understanding about the possibility of disease transmission among the refugees and their local community. We also found that the environmental and personal behavioral of hygiene apparently contributed as disease transmission among the community. The risk factor to diseases transmission was identified by mosquito (80%), rats, pets, and other insect disturbance.

Table 1. Characteristics of Subject

No	POINT OF SUBJECT	QUANTITY
A	Gender	
	1) Female	22/50 (44%)
	2) Male	28/50 (46%)
B	Age	
	1) Children (1 – 17 yo)	17/50 (34%)
	2) Adult (18 – 55 yo)	33/50 (66%)
	3) Elderly (> 55 yo)	0
C	Nationality	
	1) Somalian	13/50 (26%)
	2) Yemeni	7/50 (14 %)
	3) Sudanese	5/50 (10%)
	4) Afghan	7/50 (14%)
	5) Ethiopian	3/50 (6%)
	6) Pakistani	2/29 (6,89%)
	7) Iraqi	1/50 (2%)
	8) Others	12/50 (24%)
D	Medical Record :	
	1) Diarrhea during the last 3 months	5%
	2) Diarrhea with fever	5%
	3) Drug treatment of anthelmintic	5%
	4) Hepatitis	5%
E	Vaccination :	
	1) Hepatitis A/B	5%
	2) Malaria	5%
	3) Covid-19	60%
	4) Tuberculosis (TB)	5%
F	Environmental and Personal Behavioral of Hygiene	
	1). Availability of clean water resource	70%
	2). Availability of proper toilet	80%
	3). In house mosquito nuisance	80%
	4). In house rat disturbance	5%
	5). In house pet (dog/cat)	5%
	6). In and outside fogging	15%

Even though, they reported that the puskesmas were frequently fogging the area to respond to the dengue program and vector control, nevertheless, this program was not supported by the community hygiene behavior, as we observed as to their living area such as one small room was occupied by more than 3-5 family members, hanging clothes around the house, messy trash, in house pets, etc.

The risk indicators associated with these animals are assumed communicable infectious diseases, particularly those caused by zoonotic and neglected parasites (including protista, helminths, and arthropod), and represent a major and long-term burden in disadvantaged communities; many of these pathogens are carried by animals, such as dogs and cats, and include fleas (and the pathogens they transmit) and soil-transmitted helminthes.[14] The risk of transmission from these pathogens is a real threat in this area of study.

The refugees in this area have been living in a low standard of healthcare and daily primary needs. They are no longer financially supported nor entitled for health insurance by the district health takers in accordance to have free access of health service.[2] This situation has been worsening during the current Covid-19 pandemic. It is critical for the UNHCR Indonesia to receive sustained funding to be able to deliver protection and complementary solutions to the persons of concern as the pandemic continues to pose challenges to the already limited resettlement opportunities. The resettlement conditions are just some of the important factors that may influence the health of migrants.[1]

Another issue that may threaten refugee' health condition is the declined immunization rates in countries of origin of migrants and refugees. Our data described that only 5% of refugees have been administered hepatitis A/B, malaria, and tuberculosis vaccinations from their country. During the Covid-19 pandemic, 60% of them had been vaccinated for

Covid-19 by the local health center in their recent living at Ciputat.

Several studies highlighted that migrants and refugees have lower immunization rates compared to European-born individuals. Firstly, this is due to low vaccination coverage in the country of origin. Then, several problems may limit migrants' access to vaccination such as: information on the immunization status of migrants is often lacking; migrants often refuse registration with medical authorities for fear of legal consequences; and the lack of coordination among public health authorities of neighboring countries may determine either duplications or lack of vaccine administration. Possible strategies to overcome these problems include tailoring immunization services on the specific needs of the target population, developing vaccination registers, and promoting collaboration among public health authorities.[15]

Vaccination programs for children of the refugees is also one of the main concerns in this study. Children who are either refugees themselves or have parents who are refugees often lack routine vaccinations, either because of their parents' unawareness of the vaccination programs or because of unwillingness to participate.[16]

All refugees were informed that they could opt-out of sharing their vaccination history or having their children vaccinated. Some information records are included: a list of all the children 0–14 years of age hosted at each camp and their demographic characteristics (age, sex, and nationality), any vaccines that a child had already received, and if a child had never been vaccinated or whose vaccination status was unknown.[17,18]

Result of Sample Procedures

We conducted assays for three types of samples to identify parasitic infection in our subjects. We ran the test for urine sample by dipstick urinalysis, bacterial culture; rapid test and microscopic examination for blood and stool stain while differential blood count was performed at the laboratory of Puskesmas Pisangan and Cirendeu, Ciputat, South Tangerang district. The results are described in Table 2.

Table 2. Result of Specimen Assays

No	Type of Parasite Infection	Quantity
A	Gastro intestinal infection	
	1. Helminthiasis	0
	2. Amebiasis : *Entamoeba coli*	1/50 (2%)
B	Urinary Tract Infection (Bacterial Culture):	
	1. Unspecified bacterial of negative gram (*Enterobacteriaceae*)	2/50 (4%)
C	Malaria	
	1.Plasmodium ovale	2/50 (4%)
	2.Plasmodium vivax	3/50 (6%)
	3.Plasmodium malariae	1/50 (2%)
D	Blood diffcount	
	1. Limphocytosis	2/50 (4%)
	2. Thrombocytopenia	2/50 (4%)
	3. Leucocytosis	6/50 (12%)
	4. Anemia	1/50 (2%)
E	Urinalysis	
	1. Leucocytosis	7/50 (14%)
	2. Protein ++ with excretion of 100 mg/dl (1.0)	1/50 (2%)

Result of Stool Samples

In stool samples, we found cyst of *Entamoeba coli* in one patient of child age, a 6-year-old from Afghanistan. The characteristic of sample showed normal consistency, yellowish color, and no watery or mucus in the stool. Hence, the *Entamoeba coli* was known as a normal habitant in gastrointestinal, and people who get infected

by this parasite are usually asymptomatic. The results of urinalysis and blood test of this patient were also negative for other parasites.

Several epidemiological data were reported on the prevalence of intestinal parasitic infections among refugees from Palestine, Syria, Iraq, Turkey, and other African refugees. In the Asylum Seekers Centre of Castelnuovo di Porto (one of the largest centers in Italy), 300 migrant newcomers from sub-Saharan Africa, were screened upon their arrival for protozoa and helminth eggs from March to May 2017. The results of stool analysis showed a prevalence of intestinal parasitic infections of 20.12% in migrants from West Africa and 23.40% in those from East Africa, with no statistically significant differences.[19] Nevertheless, in our study we had no evidence of any helminth infection in the stool samples of our subjects based on these origin countries. This result might be correlated by the elimination program of helminthiasis by the puskesmas in these areas which is periodically conducted every six months.

Result of Urine Samples

Dipstick urinalysis has resulted that 14% or seven subjects showed an increased titer of leucocyte (Leucocytosis) and one subject with proteinura. This result was correlated to urinary infection by bacterial. We continued the urine assay by culture media.

We detected growth colonies on MCA as well as on NA and SSA media. The subject was a 5-year-old boy from Sudan. The physical examination and urinalysis of the sample, showed normal result as well.

Another subject was detected unspecified Gram-negative of bacteria on the NA and SSA. This subject was a female aged 22 and originally from Somalia. The differential blood count had increased value of leucocyte 10.000/µL, and dipstick urinalysis showed 125 mg/dl (++) of leucocyte, protein 15(0.15)±, and urine pH 6. These two results of blood and urine were related and confirmed by the bacterial culture of *Enterobacteriaceae*.

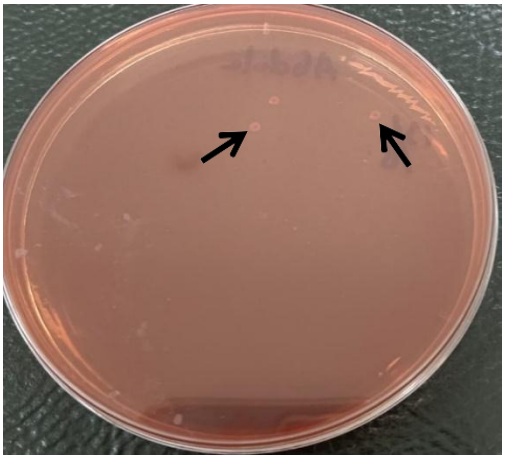

Figure 5. Bacteria growth colonies of *Enterobacteriaceae* on MacConkey Agar

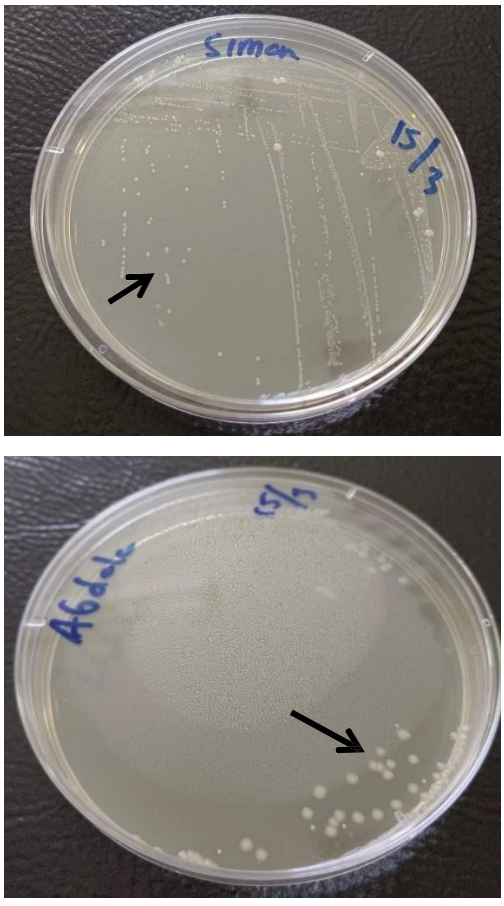

Figure 6. Bacteria growth colonies of *Enterobacteriaceae* on Nutrient Agar

These results should be confirmed by TSIA or SIM selective microbiology tests to identify species of the bacteria. But we didn't continue the test due to limited time and materials.

Result of Blood Samples

The number of people with suspected malaria confirmed by either microscopy or RDT should include both outpatient and inpatient cases. The number should include cases detected passively (attending health facilities or seen by community health workers) or actively (sought in the community); it is often useful to provide a breakdown of cases detected passively and actively. Regardless of transmission setting, any person with a positive result in a parasite-based test (microscopy or RDT), irrespective of clinical symptoms, should be considered to have a (confirmed) case of malaria.[20]

The finding of this study by microscopic blood stain showed five people with nationality from Sudan, Somalia, and Yemen, respectively, were infected by malaria; three people were infected by *Plasmodium vivax*, one with *P. malariae*, and the two others with *P. ovale*. These three species of Plasmodium were not endemic diseases nor vector transmitter inhabitants in the area of study. We suspect the infection was imported cases from their country or other previous journey from endemic areas, but no statement was declared in the medical record or questionnaire. We reported and sent the specimen to double check to the district laboratory, and recommended for the subjects to have antimalarial treatment. The treatment will prevent the subject from chronic and recurrent symptoms.

The risk for vector-borne diseases, such as malaria is very limited to non-existing in the Middle East and North African countries, but should be considered for persons originating from sub-Saharan African countries or Asia (India, Pakistan). In a range of studies, recent immigrants accounted for between 5% and 35% of reported malaria cases. Malaria in recent immigrants is often asymptomatic; the parasites may persist for up to 28 months after arrival. Vulnerable groups include pregnant women and children. In children, malaria can be easily confused with common childhood illnesses, particularly vomiting and fever, which may delay the correct diagnosis.[21]

Meanwhile, the differential blood count resulted that 12% of the samples were leucocytosis, as well as 14% of urinalysis results. This result related to the occurrence of bacterial, Plasmodium, and intestinal protozoa in the samples. Additionally, one subject was detected with a higher value of protein ++ in the urine that was suspected to associate with a certain chronic disease. We recommend the patients to be followed up to confirm the result at the higher level of the health center.

Several factors have been reported as causative agents and routes of transmission of the disease, e.g.: environmental and host behavior and factors related to the host's ability to resist infection, such as the genetic constitution and immune responsiveness. Intestinal parasites are widely prevalent in developing countries due to poor sanitation, and inadequate personal hygiene.[6]

The risk of transmission to the autochthonous population is very low, though outbreaks in the refugee population should be considered due to poor living conditions and suboptimal vaccination, not least among children. Even though we see high transmission in the refugee populations, there is very little risk of spread to the autochthonous population.[7]

Problems and Strategies

We have identified some problems for the refugees in term of accessing to the upper level of healthcare for their acute and chronic health problems. Difficulties in accessing general practice and an increased reliance on accident and emergency services for non-emergency treatment were identified, even though almost all the surveyed refugees were registered with a general practitioner.[6]

Due to the current Covid-19 pandemic, it is critical for UNHCR Indonesia to receive sustained funding to be able to deliver protection and complementary solutions to the persons of concern as the pandemic continues to pose challenges to the already limited resettlement opportunities. The Covid-19 pandemic has taken its effect in some of the income streams of the UNHCR Indonesia Private Sector Partnership. The situation has been contributing to decrease the number of refugees visiting primary health centers. Furthermore, for refugees who do not or cannot declare themselves to the statutory authority, fear of detection may discourage access to health services.[1]

The strategy on the elimination program of communal diseases, particularly from the immigrant/refugees community are required as follows: 1. Health screening upon arrival to the resettled area, 2. Vaccination to some recommended diseases. Particularly for children under 5 and elderly, 3. Provide access to primary health center and upgrading level of healthcare for specialist and psychologist/psychiatry, 4. Conducting integrative service and home-based care for elderly, malnutrition babies, mental disorder, and people suffering for a chronic disease, 5. Early diagnosis for communicable infectious diseases periodically, 6. Prompt treatment and rehabilitation program, particularly for disability limitation, 7. Monitoring to involve participation of "kader kesehatan desa" and "kesling". 8. Program evaluation included all the local health-takers, authorized people, and the communities.

Evidence-based public health measures to mitigate the health implications of migration could save a significant number of lives and reduce suffering and ill health. They are also likely to be instrumental in effectively addressing growing healthcare costs and in preventing or mitigating the negative effects of migration on health systems and societies. Nevertheless, insufficient knowledge in many areas has hampered efforts toward more effective planning and implementation of effective strategies to address migration and health. A robust multidisciplinary scientific knowledge base is therefore an essential foundation for enhancing public health practices and policy development.[5]

We address some policy considerations to improve information and to support the design of national standards and management strategies in the health and social care of refugees and asylum seekers. Policy options based on the evidence reviewed here are: improved access to services by removal of legal restrictions; provision of full health coverage for all pregnant women and for children regardless of immigration status; adoption of approaches to improve communications, such as provision of interpreters, good documentation for patients; and eventually adjustment of healthcare provision to improve service utilization, for example longer appointment times and transport provision.[6]

Through this study, we strongly suggest to improve communication and more progressive coordination between the puskesmas as the first stakeholder for the local community, and the International

NGO (such as the UNHCR, IOM, refugees, etc.) as the first takers of worldwide institutions, to conduct a strategy for screening on infectious and non-infectious diseases, provide financial resources from the local district CSR to support healthcare for the refugee and the community, and to prevent disease transmission by the vector and other intermediate host.

Following Indonesia's recent accession to the Global Compact for Safe, Orderly and Regular Migration and its role as a Global Compact "Champion Country, "there has been increased cooperation between the International Organization for Migration (IOM) Indonesia and the Government of Indonesia. To build on the success of this cooperation, IOM formulated a Country Strategy 2022–2025 for Indonesia to define a clear strategic pathway to work within the country and enhance current and future collaborations with the Government to guide its operations and strategic engagement with wider stakeholders in the country and the region.[22]

IOM will support the Government of Indonesia's engagement with inter-governmental forum to ensure coordination around migrant health and harmonization of approaches to major diseases of international importance and emerging public health threats.[22] This activity area includes the development of various standard operating procedures (SOPs), guidelines, plans and tools for the management of communicable diseases, including for detection, notification, isolation, management and referral at borders.[23]

IOM has supported the implementation of early detection and referral of cases at point of entry (PoEs) through primary and secondary screenings. Health screening procedures are adapted to the specific characteristics of an individual disease or health threat, and linked with a competent referral system connected to the national response.[23]

In addressing health issues in refugee shelters, it is essential to establish minimum standards to guide planning, provide assistance, and evaluate the work of government agencies and non-governmental organizations (NGOs).[24]

Minimum standards for refugee health services include providing comprehensive healthcare, preventing and eradicating infectious diseases, and ensuring emergency nutritional surveillance. The health services cover public health, reproductive health, and mental health support, while preventive measures target potential disease outbreaks like measles, diarrhea, smallpox, malaria, chickenpox, acute respiratory infections, and tetanus.[24] In the event of identifying an infectious disease case, prompt reporting to the Community Health Center is crucial. All stakeholders, including NGOs, are obliged to notify the District Health Service for coordinated monitoring.

In order to effectively address refugee health challenges and prevent the spread of infections, it is crucial to prioritize the management of infectious diseases. Infectious disease management essentially consists in identifying the agent cause(s) of an infection (proper diagnosis), initiating if necessary therapy against pathogens, and controlling host reactions to infection.[25] Moreover, significant advances are also included for management of sanitation in terms of controlling life cycle of vector and reservoir, proper use of chemical and biological agents against the disease's transmitter, and appropriate treatment and medication for the management of the patient and the communities.

Infectious disease management requires collaboration across sectors to

achieve more rapid, mutually beneficial and effective responses. This collaboration requires a comprehensive and strategic way of thinking about the problem of infectious diseases in order to minimize the impact. Implementation of regulatory policies in infectious disease management should involve the community and related parties, including local health officials, related stakeholders, international non-governmental organizations (INGOs), and District Health Department.

The successful elimination of an infectious disease does not entirely depend on the availability of medical infrastructures but also on the ability to comprehend the transmission of a disease and the application of control strategies together with proper implementation of logistic policies.

The effective and efficient intervention strategies are needed to prevent uncontrollable outbreaks. Some models to identify and respond to infectious disease outbreaks utilizing One Health approach are described as follows : know the principles of infection control and personal protection for responders in infectious disease management; be knowledgeable about leadership principles for the detection and response toward infectious diseases; be knowledgeable about cultural and religious issues in communities as these play a great role in infectious diseases management/control and transmission.[26] These components are very important for an effective management and control of infectious diseases outbreak.

STRENGTH AND LIMITATION

The strength of this study was originality and integrated aspects of health status of the refugees and potential transmission to the local community; and to develop awareness for collaboration between local health institution and international non-government organization (INGO) for the refugees.

The limitation of this study was lack of participation from the refugees, and restricted barrier of communication and coordination with the INGOs, such as IOM and UNHCR, to open information and collaboration for the study research.

CONCLUSIONS

Our study conclusions are that parasitic and bacterial infectious diseases remain prevalent among the refugee subjects. Based on the disease burden and the nationality of the participants, we found that Sudanese individuals had the highest prevalence of infectious diseases. Our study also revealed that mosquitoes and poor living conditions were the major potential factors contributing to the transmission of the diseases. This finding has raised awareness for the management and control strategies on the elimination program of infectious diseases, particularly from the immigrant/refugees to the local community. The plan includes health screening, vaccination, access to primary and upgraded levels of healthcare for chronic diseases, home-based care monitoring, and evaluation involving all local health providers, authorized individuals, and communities.

ETHICAL CLEARANCE

The research protocol was approved by Ethical Committee of Faculty of Medicine UIN Syarif Hidayatullah Jakarta, number:B-018/F12/KEPK/TL.00/03/2023.

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CONFLICT OF INTEREST

No conflict of interest should be stated within the study report.

AUTHOR CONTRIBUTION

Silvia Fitrina Nasution as first author and principal investigator. Hoirun Nisa as second author and contributor.

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Epidemiological, Clinical, and Occupational Characteristics of Migrant Workers Confirmed with COVID-19 at Udayana University Hospital

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has prompted Indonesian expatriates to return home. Travel history, including migrant repatriation, was thought to spread COVID-19. These factors increased COVID-19 transmission. This study examined the epidemiological, clinical, and occupational characteristics of migrant workers with COVID-19 at Udayana University Hospital. This descriptive study utilized a cross-sectional methodology. The research samples consisted of 97 migrant workers diagnosed with COVID-19 who had been treated at Udayana University Hospital from March to August of 2020, using the total sampling technique. The median age (IQR) of migrant workers is 30, and 84.53% are male. Before returning to Indonesia, many worked and visited the US (20.6%). 87.63% of respondents worked in hospitality sector, and mostly worked in 8–12-hour shifts. All respondents have insurance; most employers are provided with PPE and information regarding COVID-19 prevention. At the airport, COVID-19 screening revealed fever (70.6%) and cough (76.3%) as the most common symptoms. A 94.8% of migrant workers had no comorbidities, and 87.6% had normal chest X-rays. From this research, we discovered that amongst migrant workers, positive-COVID-19 results were mostly found in young, mobile men. Most of them worked for 8–12 hours per day, and majority worked in hospitality sector. Almost all of them did not have any comorbidities and the most common symptoms found were fever and cough. The findings suggest that male workers in their productive age with high mobility and working in the hospitality sector are at higher risk of infection.

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INTRODUCTION

The occurrence and rapid spreading of the coronavirus disease 2019 (COVID-19) pandemic have startled people worldwide. In the initial phase of this pandemic, very little information was known regarding this ailment, including its mode of transmission, incubation period, treatment, vaccination, and other features, mainly because of its rapid clinical transformations.[1,2] On December 31st, 2019, Wuhan Municipal Health Commission in China reported a cluster of pneumonia cases in Wuhan, Hubei Province. These findings were eventually identified to be caused by a novel coronavirus.[3]

Some initial cases were confirmed outside mainland China in Japan, South Korea, and Thailand on January 20th, 2020. On January 30th, 2020, the World Health Organization's (WHO) General Director declared the COVID-19 outbreak an international public health emergency and issued several recommendations regarding COVID-19 prevention and treatment.[3-5]

The surprising number of deaths caused by COVID-19 put a challenge in various sectors, especially in public health, the food system, and employment. According to the WHO, economic and social instabilities caused by the pandemic threatened millions of people to fall into poverty.[4,5] These factors urged people who had previously chosen to live, study, and work abroad to return to Indonesia.

Bali has quite a large number of migrant workers. According to data from the Center for the Placement and Protection of Indonesian Migrant Workers (BP3TKI) in Bali, there were 15,436 overseas workers from Bali.[5]

COVID-19 transmission was associated with the infected patient's travelling history. Hence, the repatriation of migrant workers can increase the risk of COVID-19 transmission between fellow workers and people in their hometowns. Migrant workers' return to their country of origin significantly impacts confirmed cases. Interactions of these repatriated migrant workers may contribute to escalating confirmed COVID-19 cases.[6] Current protocols for COVID-19 prevention mandate immigrants from foreign countries to do quarantine in facilities provided by government. The protocol applied is to do a PCR swab when you arrive, quarantine for two weeks, stay safe, and always wear a mask.[7,8] Based on those facts, the authors are interested in conducting this study to understand the epidemiological, clinical, and occupational characteristics of migrant workers confirmed with COVID-19 who were treated at Udayana University Hospital.

MATERIALS AND METHODS

Materials

Samples were taken using random sampling by accessing patients' medical records to determine which patients met the study criteria. The inclusion criteria in this study were migrant workers who previously worked in foreign countries, diagnosed with COVID-19 and treated at Udayana University Hospital, and having a complete medical record. The exclusion criteria in this study were patients whom not willing to be part of the study, or cannot be contacted by phone number recorded in the medical record. Data were collected by interviewing respondents through phone, using a questionnaire filled by the researcher.

Methods

This study is a descriptive study with a quantitative method and cross-sectional design. The target population in this study is

migrant workers who were diagnosed with COVID-19 and being treated at Udayana University Hospital from March to August 2020. The sample size was determined using a sample size application by the WHO, resulting in 97 people.

RESULTS AND DISCUSSION

Epidemiological Characteristics

This study's epidemiological characteristics include age, gender, residential regency, visited countries before returning to Indonesia, and their location when confirmed with COVID-19 (Table 1). The youngest migrant worker is 20 years old, while the oldest one is 49 years old. Thus, the median age (IQR) is 30 years.

The clinical characteristics studied were the timing of COVID-19 confirmation, symptoms experienced, comorbidities, chest X-ray results, and smoking history (Table 2). No death was recorded during treatment at Udayana University Hospital.

The occupational characteristics were occupation, country of employment, working period, working duration, information related to COVID-19 prevention from employers, personal protective equipment (PPE) availability, and insurance (Table 3).

Table 1. Respondents' Epidemiological Characteristics.

Characteristics	N	%
Age		
18-24 years	15	15.53
25-34 years	52	53.60
35-44 years	21	21.6
≥45 years	9	9.27
Gender		
Male	41	42.26
Female	56	57.7
Residential Regency		
Bangli	5	5.15
Denpasar	30	30.92
Karangasem	15	15.46
Gianyar	17	17.52
Badung	7	7.21
Buleleng	11	11.34
Jembrana	3	3.09
Lombok Tengah	6	6.18
Bandung	3	3.09
Visited Countries		
USA	20	20.6
United Arab Emirates	14	14.4
Italy	12	12.4
England	11	11.3
Qatar	8	8.3
Australia	8	8.3
Malaysia	5	5.2
Brazil	5	5.2
Spain	4	4.1
Denmark	4	4.1
Nepal	2	2.1
France	1	1.0
Barbados	1	1.0
India	1	1.0
Peru	1	1.0
Location at the time of COVID-19 diagnosis		
Airport Health Assessment	52	53.6
Medical Examination	40	41.2
Contact Tracing	5	5.2

In this study, most cases involved a male in the 25-34 years group. This phenomenon may be caused by biological differences in immunity systems between males and females and might affect the body's ability to fight infections, including SARS-CoV-2. In general, females tend to be more immune to infections than males. Potentially this theory was contributed by several factors, such as sex hormones and higher expression of coronavirus receptor (ACE 2) in males. Furthermore, higher smoking rates and alcohol consumption in males might also affect this phenomenon.

Females also tend to have more responsible behavior during the pandemic. This attitude could affect obedience to preventive manners, such as hand washing, usage of face masks, and staying at home.[9] This result was also in line with the study conducted by Ngiam (2021), which stated that some patients with COVID-19 in Singapore were healthy young workers. This study also found similarities between

Table 2. Respondents' Clinical Characteristics.

Characteristics	N	%
Timing of COVID-19 Confirmation		
March 2020	26	26.81
April 2020	18	18.56
May 2020	14	14.43
June 2020	16	16.49
July 2020	12	12.37
August 2020	11	11.34
Symptoms Experienced		
Cough	74	76.3
Fever	68	70.6
Sore throat	23	23.7
Shortness of breath	12	12.4
Headache	12	12.4
Fatigue	4	4.12
Anosmia	2	2.06
Comorbidities		
None	92	94.8
Hypertension	3	3.09
Diabetes	1	1.06
Mallory-Weiss Syndrome	1	1.06
Chest X-Ray Results		
Normal	85	87.6
Pneumonia	12	12.4
Smoking History		
Not smoking	80	82.4
1-5 years	3	3.1
5-10 years	5	5.2
>10 years	9	9.3

Table 3. Respondents' Occupational Characteristics.

Characteristics	N	%
Occupation		
Driver	4	4.12
Agriculture	4	4.12
Spa Therapist	6	6.16
Room Attendant	19	19.59
Bar Worker	12	12.4
Cook	19	19.59
Waiter	29	29.89
General Worker	4	4.12
Country of Employment		
USA	20	20.6
United Arab Emirates	14	14.4
Italy	12	12.4
England	11	11.3
Qatar	8	8.3
Australia	8	8.3
Malaysia	5	5.2
Brazil	5	5.2
Spain	4	4.1
Denmark	4	4.1
Nepal	2	2.1
France	1	1.0
Barbados	1	1.0
India	1	1.0
Working Period		
1-5 years	42	43.3
5-10 years	36	37.1
>10 years	19	19.6
Working Duration		
4-8 hours	51	52.6
8-12 hours	29	29.9
>12 hours	17	17.5
Received information about COVID-19 prevention from employers		
Yes	93	95.9
No	4	4.1
PPE provided by employers		
Disinfectant	47	48.5
Hand Soap	92	94.8
Hand Sanitizer	66	68.0
Headcap	50	51.5
Hand Glove	85	87.6
Face Shield	8	8.25
Goggles	2	2.06
Face Mask	97	100
Insured		
Yes	97	100
No	0	0
Types of Insurance		
BPJS Kesehatan	5	5.15
BPJS Ketenagakerjaan	25	25.77
Private Insurance	67	69.07
Insurance Facilitated by Employers		
Yes	38	39.18
No	59	60.82

young workers and students who returned to Hong Kong during the pandemic.[8,10]

The United States was the most visited country by respondents before they returned to Indonesia. According to a study done by Cartaxo (2021), India, United States, and Brazil were the countries with the highest risk of exposure. Rates of COVID-19 transmission in these countries were as follows: 39.5%, 19.7%, and 24.4%. The incident rates (139.7; 1449.9; 1327.6

cases per 100.000 ha) were considered medium to high.[9-12]

COVID-19 infections among these workers were confirmed mainly by detection at the airport. A study by Mouchtouri (2020) also found that 77.5% of healthcare systems that performed screening at the airport identified most imported cases.[10,12] Recently, countries worldwide implemented screening at international airports to identify import cases. This action was one of the attempts to respond to COVID-19 as an international public health emergency.[13,14-17]

In this study, fever and cough were the most prevalent symptoms among COVID-19-positive workers. Chen et al (2020)[11] also revealed similar results, namely that the most common symptoms among COVID-19 patients were fever (83%), cough (82%), shortness of breath (31%), muscle pain (11%), fatigue (9%), headache (8%), sore throat (5%), rhinorrhea (4%), chest pain (2%), diarrhea (2%), and nausea and vomiting (1%).[11]

Most respondents did not have comorbidities, possibly because they had undergone a series of medical examinations to ensure their physical and mental health before working abroad. The purpose of these examinations is to identify conditions that may pose a threat to public health. These evaluations were also advantageous for migrant workers, as they provided information about their health conditions.[12,18-20] Half of the employees who underwent chest X-ray examinations had normal results. This result was comparable to that of Yoon et al. (2020), who found that the majority of COVID-19 patients had normal chest X-rays.[13,19]

Most migrant workers treated at Udayana University Hospital had no smoking history (82.4%), while the remaining 17.6% did. Seven out of nine smokers who underwent a chest X-ray were diagnosed with pneumonia. This result was similar to what Berlin et al. (2020)[14] discovered in their study of intensive care unit patients who had previously cared for COVID-19 patients.[14,21,22] In their investigation, they compared several studies. One of these studies revealed that 108 of 913 COVID-19 patients with mild illness and 29 of 172 COVID-19 patients with severe illness were smokers. Other research revealed that three of 82 COVID-19 patients with mild illness severity and four of 58 COVID-19 patients with severe illness were smokers. According to another study, up to two of 67 patients with mild illness and three of 11 patients with severe illness were smokers.[14,23] This may appear small, but smoking was likely associated with the negative evolution and worse outcomes of COVID-19 illness. This theory was supported by the fact that tobacco smoke exposure was the primary risk factor for lung diseases, and smoking was a significant risk factor for both viral and bacterial infections virus.[24,25]

Mainly, respondents worked in the hospitality sector, such as waiter, cook, room attendant, and bar worker. In Indonesia, Bali Province was the principal recruitment place for hospitality sector workers because the people have pretty high rates of English fluency and secondary school graduate. Also, they have a good tolerance for people of various religions.[15,22]

These are several reasons why many Balinese residents, especially those in the productive age group, chose to work abroad. One of the popular occupations was working on cruise ships. In connection with this study, the jobs of most respondents have a high risk of spreading COVID-19 because it requires them to interact with many people, even having close contact with other people who may be infected with COVID-19.

The number of migrant workers in Bali was relatively high. Based on data collected by the Bali Province Government (Service Center for Placement and Protection of Indonesian Migrant Workers or BP3TKI Bali, and Manning Agency), 15,436 migrant workers in 2020 from Bali worked abroad.[5]

Based on working duration, most respondents worked 8-12 hours and >12 hours per day. This result resembled the finding in a study by Adhyatma and Hapsari (2021)[15], which stated that in the tourism industry, especially cruise ship charters, the primary purpose is to provide customers with exceptional personal experience and excellent service. These attempts were, to some extent, executed by room boys, spa therapists, and other service staff, who are on stand-by 24 hours a day.[15,25]

The services provided by yachts might seem similar to luxury hotel services. However, this system was a completely different experience for the staff because they were required to work longer shifts and shorter rest periods. Hence, they were more prone to exhaustion. Moreover, working in open seas, far from home, and for an extended period could make workers experience stress.[15,19]

Employers had conveyed information regarding COVID-19 properly, so most workers had already received information about its prevention. PPE provision for workers was the most crucial part of preventing COVID-19 transmission in workplaces, and employers have provided PPE, mainly in the form of face masks and hand soaps.[16,23,25] Furthermore, all migrant workers were already covered with or had insurance, varying from private ones or in the form of BPJS, whether paid personally or facilitated by their employers.

STRENGTH AND LIMITATION

The study was conducted online using Google Forms, which might have limited the participation of those who do not have access to digital devices, thus the population sample probably did not fully reflect the general population. Furthermore, respondents may have had different interpretations of the questions, which could lead to bias in the study's results.

CONCLUSIONS

This study sheds light on the characteristics of migrant workers treated for COVID-19 at Udayana University Hospital in Bali, Indonesia. The findings suggest that male workers in their productive age with high mobility and working in the hospitality sector are at higher risk of infection. Early detection, prompt treatment, and workplace safety measures such as providing PPE and information on COVID-19 by employers are crucial in mitigating the spread of the virus among vulnerable populations. The study emphasizes the need for continued efforts to protect these populations and prevent further virus transmission.

ETHICAL CLEARANCE

The research protocol was approved by Chairperson of the Research Ethics Commission, Faculty of Medicine, Udayana University with protocol number 021.01.1.0616.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

IMAW and CAWP contributed to the study's conception and design and critically revised the article. NPPP and CAWP were responsible for data collection, while NPPP and MF were responsible for the analysis and interpretation of the data. NPPP and MF also wrote the article, and all authors, including NPPP, IMAW, CAWP, and MF, gave final approval of the article.

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Predictive Positive Value Xpert MTB/RIF in Detecting *Mycobacterium tuberculosis* on Adult Pulmonary Tuberculosis Patients in Dr. Soetomo Referral Hospital Surabaya Indonesia

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Pulmonary Tuberculosis.

Abstract

Pulmonary tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* and transmitted via droplets. According to the WHO, TB cases in the world have reached ten million. Southeast Asia is the largest contributor, and Indonesia itself has the second-highest number in the world with an incidence of approximately 824,000 cases. The most common symptoms of active TB are cough, fever, weight loss, and night sweats. The diagnosis can be established upon the confirmation that one of the specimens contains *M.* tuberculosis. Xpert MTB/RIF provides results in less than 2 hours, whereas culture takes approximately 2-6 weeks. This research aims to evaluate the characteristics and determine the Predictive Positive Value (PPV) percentage of GeneXpert MTB/RIF, utilizing parameters derived from the gold standard examination results, namely culture. This research method is descriptive- analytic based on secondary data extracted from medical records of patients receiving care at the multi-drug resistant TB (MDR-TB) Outpatient Management at Dr. Soetomo Referral Hospital Surabaya from the period January 2019 – April 2022. The results showed that the PPV level of GeneXpert MTB/RIF in detecting the presence of *M. tuberculosis* is 90%. The diagnosis of pulmonary TB is also supported by the chest X-ray infiltrate's appearance and clinical symptoms of cough, weight loss, fever, and night sweats. Smoking and diabetes are the most common comorbid and risk factors in TB. The conclusion of this study is that the PPV for diagnosing adult pulmonary TB using the Xpert MTB/RIF is relatively high. This suggests the potential use of this method as a diagnosing tool for accurately diagnosing pulmonary tuberculosis.

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INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) and transmitted by droplets. Transmission of TB happens through the inhalation of nuclei droplets that contain the bacteria, and they will pass through the mouth, upper respiratory tract, bronchus, and alveolus.[1] The risk factors for tuberculosis are age, gender, smoking, alcohol consumption, comorbidity (diabetes, human immunodeficiency virus (HIV), hypertension, etc.), job, and poor ventilation, which will contribute to the exposure of the bacteria to the host immune system.[2]

Indonesia is the second country having the most cases, with 384 cases per 100,000 population. In Indonesia, East Java was the second province with the most TB cases, with 43,268 cases per year. Further, there are three Global High Burden Countries (HBCs) for TB, TB/HIV, and MDR/Rifampicin Resistant TB (MDR/RR TB), Indonesia, placed in a section that includes in all three sections of HBCs.[3] Tuberculosis is classified by the location (pulmonary or extrapulmonary), treatment history (new or relapse cases), medication sensitivity , and the HIV.[4] According to the WHO, pulmonary cases account for 90% of tuberculosis cases.[3]

Distinguishing tuberculosis involves categorizing it as either bacteriologically confirmed (with a positive GeneXpert/AFB/Culture test) or clinically confirmed (symptoms, risk factors, and chest X-ray support, but lacking bacteriologic confirmation). Typical symptoms include persistent cough, prolonged fever, night sweats, weight loss, and hemoptysis. GeneXpert serves as the primary screening tool for tuberculosis suspects, and a positive result requires confirmation through the gold standard culture test.[4]

Even though the culture method remains the gold standard for diagnosing tuberculosis, it has drawbacks, including test complexity and prolonged turnaround time (approximately 6-9 weeks).[5] In 2013-2014, Indonesia introduced GeneXpert, which based on nucleic acid amplification (NAAT), delivers results within two hours and employs molecular technology to identify rifampicin resistnce.[6,7] Targeting the *rpoB* gene, which plays a role in *Mycobacterium tuberculosis* replication and serves as a potential rifampicin target, allows simultaneous detection of the bacteria and rifampicin resistance through PCR technology.[8,9] Both culture methods and GeneXpert demonstrate high accuracy, with culture methods reaching 90% sensitivity and 100% specificity, and GeneXpert achieving 81.8% sensitivity and 96.5 specificity. [10,11] The aim of this study is to evaluate the accuracy (Predictive Positive Value) of GeneXpert MTB/RIF.

MATERIALS AND METHODS

Population and Sample

The data used in this research were secondary data, obtained from medical records of adult patients with lung tuberculosis multi-drug resistance at the Dr. Soetomo Surabaya General Hospital from the period January 1st, 2019, until April 31st, 2022. The sample used in this research is all medical records of adult MDR-TB patients (above 18 years old) that meet the inclusion criteria, including complete medical records.[12] The exclusion criterion was inpatient management. The total number of patients in the MDR-TB outpatient management is 255; the number of patients that meet all the criteria is 197.

Methods

This research design is an observational retrospective study, opting for a descriptive analytic approach to assess the high accuracy (predictive positive value) of GeneXpert as a screening for tuberculosis. The data were analyzed using Microsoft Excel version 16.65, applying the predictive value formula—calculated as true positives divided by the sum of true positives and false positives.

RESULTS AND DISCUSSION

The research results showed that the majority of patients with MDR-TB within the productive age group were between 18-45 years old. This finding aligns with the statement from the WHO that the highest incidence of cases falls within the 25-34 age range.[13] The risk of exposure of Mtb through pollution and smoking was higher in population above 15 years old. The increased risk for young people might be mainly because of the continued transmission within the community.[14] Based on this research; men are more likely to have tuberculosis. The WHO also stated that males have a 1.6-fold a higher risk than females. There are several factors that can cause this gender gap, such as biological differences, and men are also likely to have the risk factor associated with TB exposure. [13,15] At the MDR-TB outpatient department Dr. Soetomo Surabaya General Hospital, the majority of cases involve new cases, followed by relapse cases. Several hypotheses exist regarding the prevalence of relapse, including the likelihood that the immune systems of recently recovered individuals have not fully recovered. Other than that, individuals who have not fully completed treatment often reside in high-risk TB exposure environments, potentially explaining the elevated risk of bacterial reactivation among these patients,[16] as shown in Table 1.

Table 1. Profile and diagnosed status of adult patients at MDR-TB outpatient department Dr. Soetomo Surabaya General Hospital, January 2019 – April 2022.

Predictor	Amount	Percentage
Age		
18-45	115	58.38
46-59	59	29.95
>60	23	11.67
Gender		
Male	101	51.27
Female	96	48.73
Diagnosed		
New cases	85	43.14
Relapse cases	60	30.45
Loss to follow-up	18	9.13
Failed treatment	18	9.13
Unstandardized treatment	5	2.53
Non-DOTS*	9	4.56
Unknown	2	1.02

*DOTS, directly observed treatment shortcourse

Risk factors and comorbidities such as smoking, alcohol consumption, diabetes, HIV, and immunosuppressive therapy significantly impact the treatment outcome and mortality rates of TB patients.[17] Table 2 shows that diabetes is the predominant comorbidity among the subjects in this study, consistent with previous research indicating a threefold increased risk of TB infection in individuals with diabetes. Alveoli play a crucial role in TB infections and their

replication.[18,19] Diabetes mellitus, with its potential to damage mediated cells and poor glycemic control, adversely impacts cytokine response, modifying the defense mechanisms in alveolar macrophages. Hyperglycemia disrupts the recruitment of neutrophil, movement of the monocyte, and phagocytosis of alveolar macrophage. Inactive T-Helper cell activation further hampers the release of the antigen-specific interferon gamma. Pulmonary microvasculature mutations and micronutrient deficiency contribute to the invasion and formation of TB.[20]

Another common comorbidity is HIV. The main effect of immunosuppressants on HIV is the loss of the CD4, which is the biggest contributor to establishment of TB. The increase of TNF-α could elevate the risk of developing TB. Decreasing apoptosis, increasing necrosis of macrophage from the infected HIV , and impeding the development of specific immune response may be associated with the immune system in HIV-infected individuals, which makes them susceptible to TB infections.[21]

Smoking and alcohol consumption are the main risk factors with infections such as TB.[19,21] Smoking has a significant role in the pathogenesis of tuberculosis, especially in ciliary dysfunction, decreasing immune system, and the damage of macrophage immune system that could be the underlying cause of TB infections. Smoking also decreases the production of IL-12, which affects infection on immunocompetent individuals.[19] Alcohol consumption adversely affects the immune system, which increases the risk of tuberculosis infection and the reactivation of latent tuberculosis. While macrophages play crucial role in eliminating various bacteria, alcohol consumption may compromise the responsiveness of alveolar macrophages to new pathogens.[22]

Table 2. Comorbidities and risk factors of adult patients with MDR-TB at outpatient department Dr. Soetomo Surabaya General Hospital January 2019 – April 2022.

Predictor	Amount	Percentage
Comorbidities		
Diabetes Mellitus	70	35.53
Gastric problem	5	2.54
Hypertension	9	4.57
HIV	7	3.55
Malnutrition	1	0.50
None of the above	92	46.70
Risk Factors		
Smoking	71	36.04
Alcohol consumption	27	13.70
None of the above	99	50.25

Table 3 shows the X-ray manifestations in tuberculosis patients. While chest X-rays provide a rapid indication of pulmonary tuberculosis, their weakness lies in imaging pulmonary infections, as various infections have similar signs and symptoms.[23] In Indonesia, the regulation by the Ministry of Health stated that every patient suspected of tuberculosis with negative bacteriologic test result should undergo chest X-ray to confirm the diagnosis. If the chest X-ray leads to tuberculosis, it will declare "tuberculosis clinically confirmed."[4] In another study, it was stated that the common chest X-ray on suspect tuberculosis was infiltrate,[24] which was in line with this study, whose chest X-

ray examination was done when the patients first came in.

Table 3. The chest X-ray of adult patients in MDR-TB outpatient department at Dr. Soetomo Surabaya General Hospital January 2019 – April 2022.

CXR	Amount	Percentage
Cavity	56	28,43
Infiltrate	117	59,39
Nodule	2	1,01
Miliary	2	1,01
Fibrosis	95	48,22
Fibrothorax	4	2,03
Pleural effusion	6	3,00
Atelectasis	10	5,07
Consolidation	9	4,56

The prevalent clinical features observed in TB patients in this study are cough, weight loss, fever, and night sweats. Cough, particularly common in lung tuberculosis, plays a significant role in its transmission, as shown in Table 4.[25] Weight loss is a frequent occurrence in TB patients, attributed to the production of inflammatory mediators, ultimately leading to leptin suppression in the progression of TB.[26] Fever and night sweats are often interconnected, with secondary inflammation by the tubercle contributing to fever. Although the exact mechanism of the rising body temperature, mainly at night remains unclear, it may be a factor in the occurrence of night sweats.[27]

Table 4. Clinical manifestation of adult patients at MDR-TB outpatient department Dr. Soetomo Surabaya General Hospital January 2019 – April 2022.

Clinical manifestation	Amount	Percentage
Cough	188	95.43
Night sweats	89	45.18
Blood cough	47	23.86
Dyspnea	79	40.10
Weight loss	141	71.57
Fever	85	43.15
Chest pain	50	25.38

*One patient could have more than one clinical manifestation.

The predictive positive value indicates the percentage of people who have a positive result on the test and are confirmed to have the disease. A predictive positive value can be obtained by dividing the true positive by the addition of true positive and false positive.[28] A false positive percentage of 0.08% remains in this sample, indicating that, although the gold standard yielded negative results, the Xpert MTB/RIF produced a positive result. The CT Value result of Xpert may have been in the "low or very low" range or around 28.3, and the false positive may have resulted from the lack of bacterial growth on the culture technique.[29] Another contributing factor to the persistence of false positive results is the inability to culture *Mycobacterium tuberculosis* DNA, which may exist extracellular or in a non-intact cell. Consequently, the manufacturer of Xpert MTB/RIF recommends that Xpert MTB/RIF should be used in conjunction with the gold standard.[30] According to this study, 90% of patients with a positive result on Xpert MTB/RIF were confirmed to have the condition listed in Table 5. This result is consistent with the previous study. Permatasari et al. reported that the predictive positive value of Xpert MTB/RIF is 90%, whereas Allahyartorkaman et al. reported that it is 83.9%.[10,31]The ability of the positive predictive value on Xpert MTB/RIF to identify *Mycobacterium tuberculosis* is

crucial for diagnosing TB. Additionally, because it may provide information on rifampicin resistance, and may serve as the basis to precisely administer the treatment and as early as possible .[32]

Table 5. Culture test results of adult patients at MDR-TB outpatient department Dr. Soetomo Surabaya General Hospital January 2019 – April 2022.

	GeneXpert (+)
Culture (+)	180
Culture (-)	17

*Culture test for *Mycobacterium tuberculosis* and GeneXpert for MTB detected and rifampicin resistance.

STRENGTH AND LIMITATION

The strength of this study lies in the use of Xpert MTB/RIF as a diagnostic tool in Indonesia. However, the limitations of this study were due to its retrospective method and following the government program, where negative results in GeneXpert are not further confirmed through culture or the gold standard.

CONCLUSIONS

The predictive positive value of the diagnosing method using the Xpert MTB/RIF is 90% for adult patients, which is relatively high; therefore, this result potentially supports the applicability of the Xpert MTB/RIF as a diagnosing tool for accurately diagnosing pulmonary tuberculosis.

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CONFLICT OF INTEREST

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

ETCHICAL CLEARANCE

All the protocol and the use of medical records for the data on this research are approved by Dr. Soetomo Surabaya General Hospital ethics committee (Ref. No.: 1055/LOE/301.4.2/1X/2022).

AUTHOR CONTRIBUTION

Every author has equally contributed to this research, from the design to the drafting and revision, and given their final approval of the article.

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Review Article

IJTID

(INDONESIAN JOURNAL OF TROPICAL AND INFECTIOUS DISEASE)

Scientific Journal of Tropical and Infectious Disease

Effectiveness of Vaccines Booster Against Infection, Severe Disease and Death Related to COVID-19 : A Systematic Review and Meta-Analysis

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Abstract

COVID-19 is an infectious disease as a result of a type of corona virus. COVID-19 is now a pandemic affecting many countries. This study aims to know the effectiveness of booster vaccines to reduce the severity of illness, confirm infection, hospitalization, death in humans infected with COVID-19. The specific purpose was to analyze the severity of COVID-19 disease in humans by booster and without booster. The design of this study was a systematic review and meta-analysis based on observational studies, published in databases such as PubMed, Embase, MedRxiv, Nature and Scopus. In the search for articles, the limitations of 2021 to 2022 are used. This research was analyzed quantitatively through the Review Manager 5.4.1 program. Study was taken from 13 journals that met the criteria for a meta-analysis. With the population aged over 18 years, and using the type of vaccine BNT162b2 or mRNA. The population of this study came from Israel, Italy, England, Qatar, Brazil, Turkey, Puerto-Rico, Northern Bangkok, Vicinities and Thailand. Significant results were obtained for each outcome. The OR values of BNT162b2 booster vaccine against confirmed infection OR 0.16 (95% CI 0.06 – 0.45), against symptomatic disease 0.22 (95% CI 0.11 – 0.44), against asymptomatic disease OR 0.72 (95% CI 0.69 – 0.74), against hospitalization OR 0.12 (95% CI 0.06 – 0.22), against severe disease OR 0.15 (95% CI 0.07 – 0.33), and against death OR 0.10 (95% CI 0.04 – 0.31). Administration booster vaccines are effective in reducing infection rates, disease severity, and deaths from COVID-19.

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INTRODUCTION

In December 2019, a local epidemic of COVID-19 was reported in Wuhan, China after it was caused by SARS-CoV-2. Since 2020, COVID-19 has outspread globally and is now affecting many countries. Over 346 million confirmed cases and beyond 5.5 million deaths have been announced throughout the world as of January 23, 2022. The death rate from COVID-19 in Indonesia reached 147,342 of the total positive cases of COVID-19, with the Case Fatality Rate (CFR) reaching 3.15% as of February 24, 2021. Carrying out a complete dose of vaccination is one of the prevention strategies for dealing with the transmission of the SARS-Co-2 virus.[1]

Beyond 409 million confirmed infections and 5.8 million deaths were reported universally in February 2022. At the geographical level, the Western Pacific Region showed a 19% improvement in new weekly cases, whereas the rest of the world saw a drop: Southeast Asia (37%), and the Americas (33%). the two most populous regions in the world (32%), and region of the Eastern Mediterranean (12 %).[2]

The pandemic has been going on for a couple years, but so far, no effective therapy has been found. The therapy used for COVID-19 is an antiviral that is not specific for the SARS-CoV-2 strain. The existing therapies are divided into several groups, namely viral protease inhibitors such as lopinavir, RNA virus inhibitors such as favipiravir which is commonly used for influenza therapy, immunomodulatory inhibitors of the virus, improvement of host immunity, inhibitors of fusion of virus with host cells such as bromhexine, and inhibitors of entry. virus in host cells such as convalescent plasma therapy.[3] This convalescent plasma therapy still raises the pros and cons of experts. According to Joyner et al. (2020)[4] covalent plasma donor therapy is still less significant for medication of COVID-19 patients.[4]

However, based on research by Salazar et al (2020), this therapy increased the clinical improvement of patients.[5] In addition, COVID-19 patients are also given symptomatic therapy only to treat existing symptoms. Therefore, a strategy is needed in dealing with the transmission of the SARS-CoV-2 virus, namely through prevention.

The infection cases have risen since the appearance of Omicron, the new variant of SARS-CoV-2. There are 26 to 32 variants found in the spike region of Omicron, some of which are worrisome and may be a source of immune escape and high communicability. The situation remains uncertain, however. Due to the presence of mutations that can confer the potential for evading immunity as well as increased transmissibility, Omicron likely has the potential for wider global spread. Given these characteristics, it is possible there will be spikes in COVID-19 cases in the future, depending on a number of factors, which could lead to the major consequences. The overall global risk associated with Omicron's new variant of concern is considered very high.[6] Omicron's global resurgence has raised serious concerns. In countries with good vaccination coverage, Omicron has led to a fresh wave of illnesses. Around 30 mutations in Omicron are identical to the earlier variation of concerns, which might reduce VE. As a result, the appearance of Omicron is anticipated to pose a serious threat to public health and might change the course of COVID-19 vaccinations.[7]

Previous study shows that 56% of the 210 persons in this cohort research who had seroconversion evidence during a regional Omicron variant increase denied having recently having had an Omicron

variant infection.[8] The evidence in the literature demonstrates that these eight vaccinations are quite successful in defending the populace against deadly illnesses, yet there are some concerns about their safety and side effects. Additionally, booster injections and immunizations tailored to certain variants would be necessary.[9]

One of preventive actions that could be taken in order to decrease infection cases is with a booster dose vaccine. So far, the relationship between booster vaccines on the incidence of confirmed infection, death, hospitalization and disease severity is still unknown. Furthermore, the data regarding the impact of booster vaccines are considered to be lacking. This study aims to determine the effectiveness of booster vaccines on disease severity, confirmed infections, hospitalizations and deaths.[1]

MATERIALS AND METHODS

Search Strategy

This study collects retrospective or prospective case-control and cohort observational studies, which will then synthesize data and/or analyze data from these studies to create a meta-analysis produce meta-analysis and/or systematic review. This analysis uses the priority report item recommendations in the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement as a reference for the method procedure looking[7]. The research sources came from the literature obtained from the online databases PubMed (n = 50), Medrxiv (n = 508), Embase (n = 176), Nature (n = 50), and Scopus (n = 44). The data collection technique in this research is scientific research from English-language journal publications using the keywords “Vaccine” AND (“booster” OR “third dose”) AND ('COVID-19” OR “Sars-CoV-2”) AND (“severe illness" OR "severe disease" OR "hospitalization" OR "mortality" OR "death"). Article searches used the 2021 to 2022 limits. Based on the search results using the relevant keywords on a predetermined search engine, then the articles were chosen based on its abstract and title then selected based on the inclusion and exclusion criteria. Next was data grouping based on the variables to be discussed and then data synthesis to obtain a systematic study related to the effectiveness of booster vaccines against disease severity and mortality due to COVID-19.

Study Selection

Articles were first reviewed by three independent authors (MK, MGP, PPS) based on the title and abstract. All inappropriate publications were removed. Then the full text of the remaining articles was reviewed. Three independent reviewers evaluated articles as potentially suitable. After that, the reviewers discussed until the suitability of the article to be used was obtained.

Eligibility and Inclusion Criteria

The research design used in this study is observational research and includes cross-sectional, case-control, and both retrospective and prospective cohorts. The population studied were all ages that had been received booster vaccinations; studies with COVID-19 booster vaccine interventions, studies with a population comparator that had not received a booster vaccine (non-booster group), research on the vaccine’s booster effectiveness against severe disease, confirmed infection, and mortality related to COVID-19, and studies in English were all included.

Exclusion Criteria

Studies that are not observational, systematic review and/or meta-analysis,

studies that go into duplication, vaccine effectiveness data that do not match the outcome, and studies in a language other than English.

Data Extraction

The data extraction consist of author's name; year of publication; research design; research location; the population under study; total number of samples and gender; average age of the sample with standard deviation; statistical analysis used in the literature; types of primary vaccine; the timing of the booster vaccine is different from the second vaccine; types of booster vaccines.

Quality Assessment

Evaluation of the quality of studies from the literature collected was using The Newcastle-Ottawa Scale (NOS) which assesses through three major parts, namely: selection, comparability, and exposure. In this study, the assessment based on NOS scores is [10] : (1) Good quality: 7-9 points; (2) Fair quality: 4-6 points; (3) Low quality : 0-3 points.

Analysis

After data extraction, data processing in the Review Manager (RevMan) version 5.4 program was performed. A random effect model was used to approximate the combined odds ratio (OR) for the incidence of confirmed infection, high morbidity, and COVID-19-related death attributable to the booster and non-booster groups with 95% confidence intervals. Then heterogeneity between the statistically tested studies test 2 and I2, with $p<0.10$ and 50%, sequentially, was reviewed as an indicator of diversity. The effectivity of vaccine is marked as the OR, along with the 95% confidence interval.

RESULTS AND DISCUSSION

Characteristics of Included Studies

Researchers conducted a search for predetermined keywords, through the PubMed, Medrxiv, Embase, Nature, and Scopus databases. After conducting a search, the researcher obtained a total of 828 available literatures. The researcher conducted an examination with the same title in the 386 studies, and excluded as many as 34 duplicates, so that there were 794 studies without the same title. Then the first stage of screening was carried out to find the availability of full text from the literature, and it was found that one study was not available in full text, so that there were 793 studies left. In the second stage of screening, the researchers carried out the first stage of the screening process, and obtained 760 titles and abstracts that did not match so that there were 33 studies remaining. The researcher then continued with the third stage of the screening process, and 17 studies were excluded because the desired outcome was not obtained, so that the remaining 16 could be included in the systematic review and 13 could be included in the meta-analysis. The results of this filtering process are shown in Figure 1. The characteristics of sixteen observational studies are summarized in Table 1.

A total of 828 publications were screened for Effectiveness of Booster Vaccines COVID-19. A total of 13 journals met the criteria for the meta-analysis. All included studies were reported during the 2021 publication year. All journals are in English, with the population aged over 18 years, and using the type of vaccine BNT162b2 or mRNA. The population of studies came from Israel, Italy, England, Qatar, Brazil, Turkey, Puerto-Rico, Northern Bangkok, Vicinities, and Thailand. Significant results were obtained for each

outcome. The studies were used to explain the difference in outcome between the population that was given a booster and that was not given a booster. Six studies explain the effectiveness of boosters against confirmed infection, as many as five journals explain the effectiveness of boosters against symptomatic disease. Three studies explain the effectiveness of boosters against asymptomatic disease, six studies explain s the effectiveness of boosters against hospitalization, seven studies explain the effectiveness of boosters against severe disease and seven studies describe the effectiveness of boosters against death.

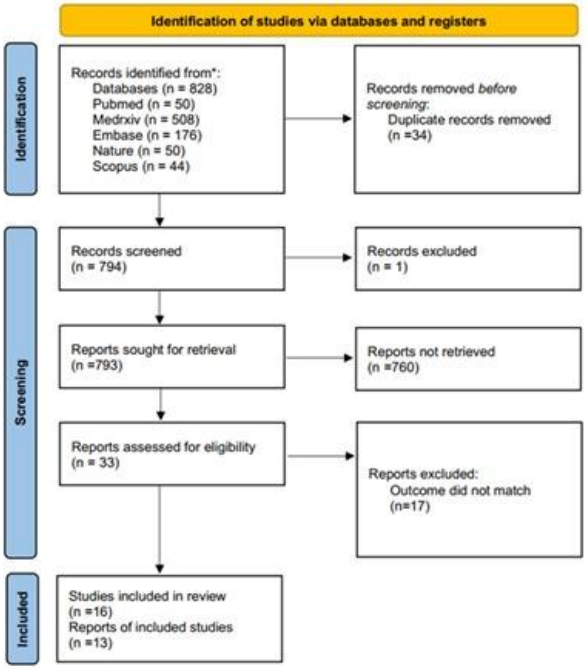

Figure 1. Literature screening chart using PRISMA 202011. Of the 16 studies included in the inclusion, a systematic review was carried out and 13 were subject to a meta-analysis.

Table 1. Characteristics of the included studies and the results.

Author & Year	Study Location	Study Design	Population/Age Range	Duration time of Research	Type of Vaccine Booster/Result
Muhsen et al.[11]	Israel	Observational retrospective cohort study	41,623 participants/Observed group: >60 years old, Control group: all ages	July-September 2021	BNT162b2 **Symptomatic disease**: Incidence Rate Ratio (IRR) 0.29 ; relative rate reduction 71% **Hospitalization**: IRR 0,20; relative rate reduction 80% **Death**: decrease from 0.3 per 1000 population in week 34 to 0.1 per 1000 population in week 36

Spitzer et al.[12]	Tel Aviv, Israel	Prospective cohort study	1,928 participants/Aged 18 years old and older	August 8 and 19 - September 20, 2021.	BNT162b2 **Confirmed infection**: adjusted Hazard Ratio (aHR) 0.07 (95% CI, 0.02-0.20); 44 participants (5 booster group, 39 non-booster group) **Symptomatic disease**: 31/44 (70.5%)
Mattiuzzi and Lippi[13]	Italia	Case-control	5.2 million participants/Older (>60 y.o), fragile people, booster vs. non-booster group	October-December 2021	BNT162b2 **Confirmed infection**: Vaccine Effectiveness (VE) 65% **Hospitalization**: VE 69% **Severe disease (ICU admission)** : VE 67% **Death**: VE 97%
Mattiuzzi and Lippi[13]	Italia	Case- control	1,549,747 participants/60 years old and older	October-December 2021	BNT162b2 **Confirmed infection**: 75% lower risk **Hospitalization & ICU admission**: 82-83% lower risk **Death**: 81% lower risk
Berec[14]	Czech Republic	Retrospective Cohort study	10,701,777 participants/Various age	March 1 2020 (first detected case) - November 20 2021	Comirnaty, Spikevax **Confirmed Infection**: Comirnaty booster: VE 92% **Hospitalization**: Comirnaty booster: VE 95%; Spikevax booster: 98% **Death**: VE Comirnaty booster: 97%; Spikevax: close to 100%
Spitzer et al.[15]	Israel	Observational retrospective cohort study	5,065,502/≥ 60 years old	July 30 - October 10 2021	BNT162b2 **Confirmed infection**: Rate Ratio 12.3 (95% CI 11.8-12.8) **Severe disease**: Rate Ratio 17.9 (95% CI 15.1-21.2) **Death**: Rate Ratio 14.7 (95% CI 10.0-21.4)
Bar-On[16]	Israel	Observational restrospective cohort study	1.137.804/≥ 60 years old	July 30 - August 31 2021	BNT162b2 **Confirmed infection**: aHR 11.3 (95% CI 10.4012.3) **Severe disease**: aHR 19.5 (12.9-29.5)

Andrews et al.[17]	England	Test-Negative Case-Control design	271,747/>50 years old	September 13 - October 29, 2021	BNT162b2 **Symptomatic disease**: VE 87.4% (primary vaccine ChAdOx1s: Vaxzefria, Astra Zeneca); VE 84.4 % (primary vaccine BNT162b2 (Comirnaty, Pfizer-BioNTech)
Abu Raddad[18]	Qatar	Retrospective cohort studies	2,232,224/All ages	January 5, 2021 and January 9, 2022	BNT162b2, mRNA-1273 **Symptomatic disease**: BNT162b2: aHR 0.50 (95% CI 0.47-0.53); mRNA-1273 aHR 0.49 (95% CI 0.43-0.57)
Silva, T C et al,[19]	Brazil	Test-negative design case control	14 million/All ages	18 January to 11 November 2021	BNT162b2 **Confirmed infection**: VE 92.7% **Hospitalization**: VE 97.3%
Andrews et al.[17]	England	Test-negative case-control design	aged 18 years and over	13 September 2021 to 5 December 2021	BNT162b2, mRNA-1273 **Symptomatic infection**: VE 94-97% **Hospitalization**: VE 99.2% (primary vaccine ChAdOx1s); VE 98.6% (primary vaccine BNT162b2) **Death**: VE 97.8% (primary vaccine ChAdOx1s); VE 98.7% (primary vaccine BNT162b2)
Barda et al.[20]	Israel	Retrospective cohort studies	1,158,269/37-68 years old	July 30, 2020, and Sept 23, 2021	BNT162b2 **Hospitalization**: VE 03% **Severe disease**: VE 92% **Death**: VE 81%
Uzun et al.[21]	Turkey	Retrospective cohort studies	1,401/General populations	August 1-August 10 2021	BNT162b2 **Hospitalization:** lower hospitalization in booster group (11/1,401 = 0.8%)
Robles-Fontán[22]	Puerto-Rico	Case Control	540,140/General populations	December 2020 - November 28, 2021	BNT162b2, mRNA-1273 **Hospitalization:** VE 89% **Death:** VE 94%

Sritipsukho[23]	Northern Bangkok	A test-negative case-control design	3,353/Adults ≥18 years old	25 July 2021 to 23 October 2021.	BNT162b2, ChAdOx1s **Confirmed infection**: VE 98% (BNT162b2 booster); VE 86% (ChAdOx1s booster) **Asymptomatic disease**: 3/1118 (ChAdOx1s booster); 0/1118 (BNT162b2 booster) **Hospitalization**: **Mild**: 9/1118 (ChAdOx1s booster); 1/1118 (BNT162b2 booster) **Moderate**: 0/1118 (ChAdOx1s booster); 0/1118 (BNT162b2 booster) **Severe disease**: 0/1118 (ChAdOx1s booster); 0/1118 (BNT162b2 booster) **Critical**: 0/1118 (ChAdOx1s booster); 0/1118 (BNT162b2 booster)
Arbel et al.[24]	Israel	Retrospective cohort studies	758,118/≥ 50 years	August 6-September 2021	**Death**: aHR 0.10 (95% CI 0.07-0.14; P<0.001)

Quantitative Analysis of the Effectiveness of Booster Vaccines Against Deaths

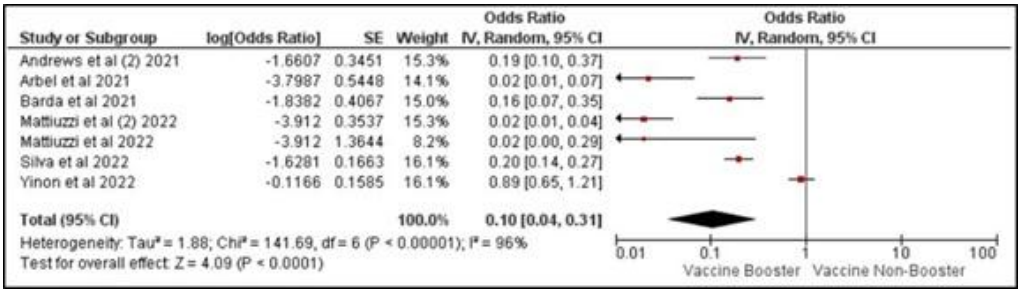

Figure 2. Forest Plot with outcome against confirmed infection by BNT162b2 vaccine booster.

From the six studies that we used to assess the BNT162b2 effectiveness of booster vaccine to prevent infection of SARS CoV-2, the results were significant with OR 0.16 (95% CI 0.06 – 0.45) and P = 0.0004 (Figure 2). The heterogeneity of the six studies was above 50% (I2: 100%, P < 0.00001), which means there was a high level of heterogeneity. So, using the random effect model (REM) analysis model , from the results of the funnel plot (Figure 3), it appears that there are four journals on the left and two on the right, so it looks asymmetrical due to bias.

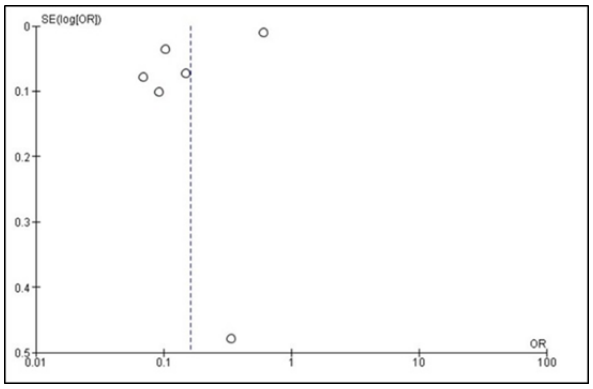

Figure 3. Funnel Plot with outcome against confirmed infection by BNT162b2 vaccine.

Quantitative Analysis of the Effectiveness of Booster Vaccines Against Symptomatic Disease

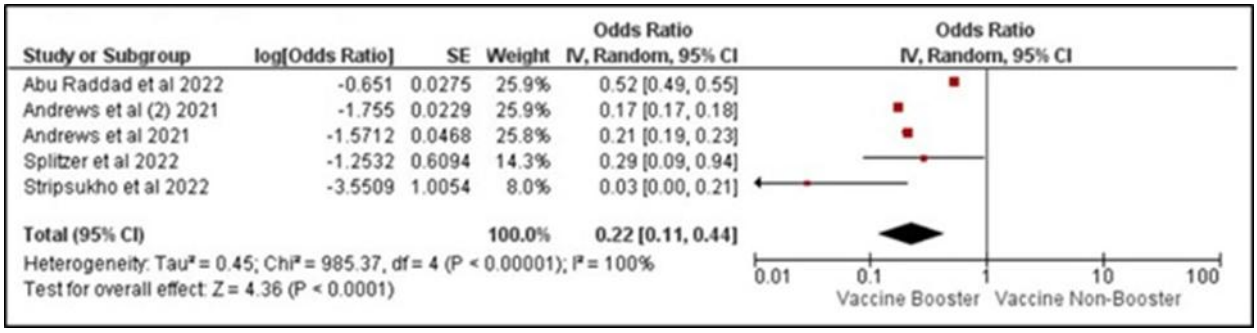

Figure 4. Forest Plot with outcome against symptomatic disease by BNT162b2 vaccine booster.

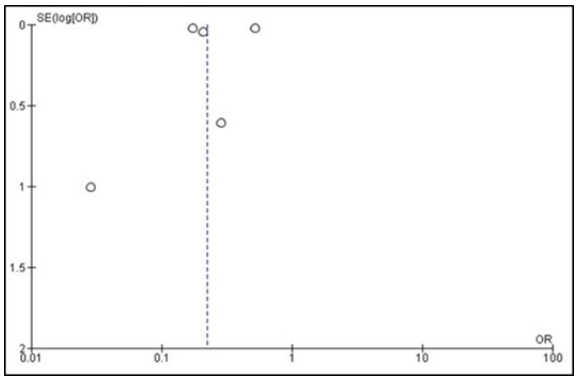

Figure 5. Funnel Plot with outcome against symptomatic disease by BNT162b2. vaccine

For this study, we used five publications to assess the BNT162b2 booster vaccine effectiveness to prevent symptoms for SARS-CoV-2 infection. The results were significant with OR 0.22 (95% CI 0.11 – 0.44) and P < 0.0001 (Figure 4). The heterogeneity of the five studies was above 50% (I2: 100%, P < 0.00001), which means it had a high level of heterogeneity. So, using the random effect model (REM) analysis model from the results of the funnel plot (Figure 5), it appears that there are three journals on the left side and two on the right, so it looks asymmetrical due to bias.

From the three studies that we used to assess the BNT162b2 booster vaccine effectiveness to prevent asymptomatic infection of SARS CoV-2, the results were significant with OR 0.72 (95% CI 0.69 – 0.74) and P < 0 .00001 (Figure 6). For three studies, heterogeneity was below 50% (I2: 0%, P 0.57), which means they had a low level of heterogeneity.

Quantitative Analysis of the Effectiveness of Booster Vaccines Against Asymptomatic Disease

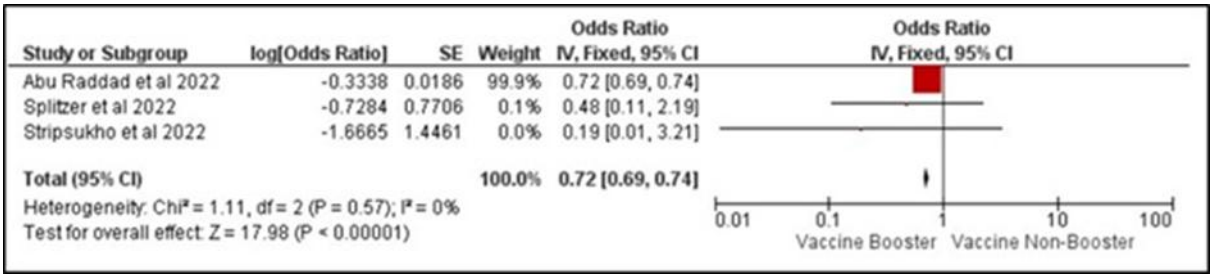

Figure 6. Forest Plot with outcome against asymptomatic disease by BNT162b2 vaccine booster.

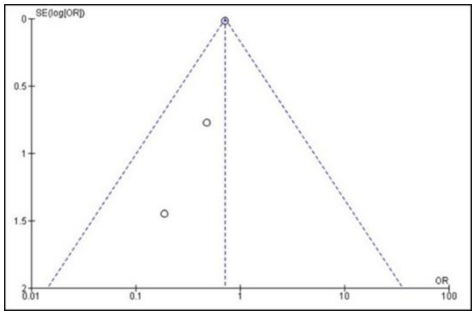

Figure 7. Funnel Plot with outcome against asymptomatic disease by BNT162b2 vaccine booster.

had a low level of heterogeneity.

So. using the fixed model (FM) analysis model, from the results of the funnel plot, it appears that there are two journals on the left side which appear asymmetrical due to bias (Figure 7).

Quantitative Analysis of the Effectiveness of Booster Vaccines Against Hospitalizations

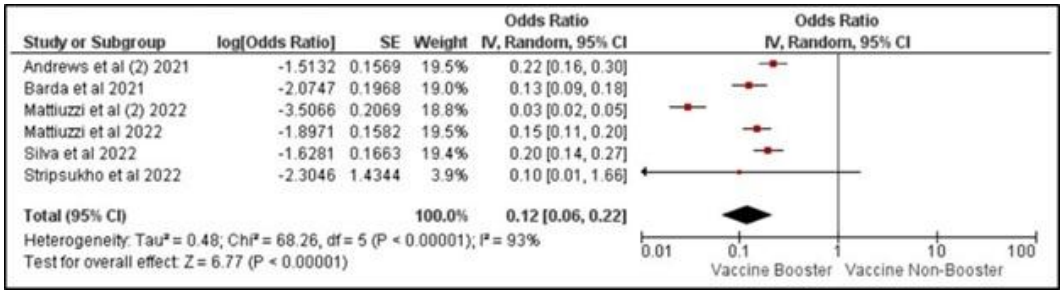

Figure 8. Forest Plot with outcome against hospitalization by BNT162b2 vaccine booster.

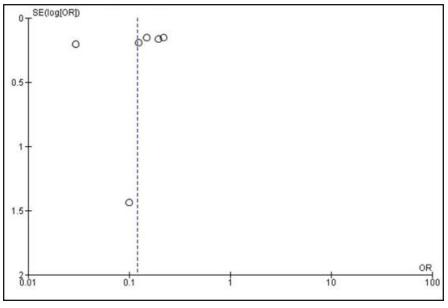

Figure 9. Forest Plot with outcome against hospitalization by BNT162b2.

From the six studies that we used to analyze the BNT162b2 booster vaccine effectiveness to prevent hospitalization, the results were significant with OR 0.12 (95% CI 0.06 – 0.22) and P <0.00001 (Figure 8). From six studies, heterogeneity was above 50% (I2: 93%, P 0.00001), which means there was a high degree of heterogeneity.

So, using the random effect model (REM) analysis model, from the results of the funnel plot, it appears that there are two journals on the left side, one in the middle, and the other three on the right side, so it looks asymmetrical due to bias (Figure 9).

Quantitative Analysis of the Effectiveness of Booster Vaccines Against Severe Diseases

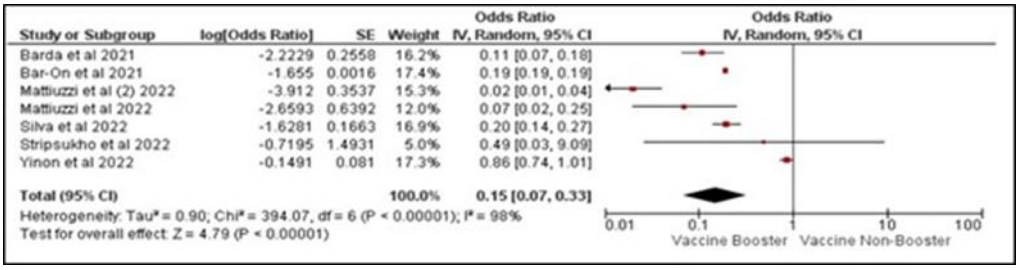

Figure 10. Forest Plot with outcome against severe disease by BNT162b2 vaccine booster.

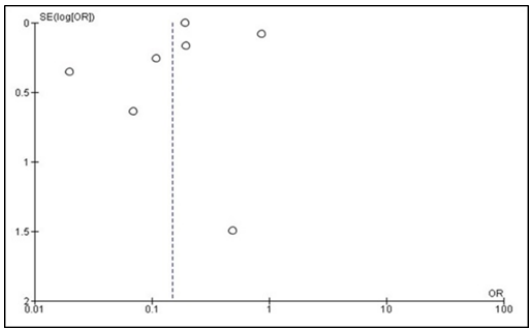

Figure 11. Funnel Plot with outcome against severe disease by BNT162b2 vaccine booster.

From the seven studies that we used to analyze the BNT162b2 booster vaccine effectiveness to prevent severity, the results were significant with OR 0.15 (95% CI 0.07 – 0.33) and P < 0.00001(Figure 10). The heterogeneity of the seven literatures was above 50% (I2: 98%, P 0.00001), which means there was a high degree of heterogeneity. So, using the random effect model (REM) analysis model, from the results of the funnel plot, it appears that there are three journals on the left side, and the other four on the right side, so it looks asymmetrical due to bias (Figure 11).

Quantitative Analysis of the Effectiveness of Booster Vaccines Against Death

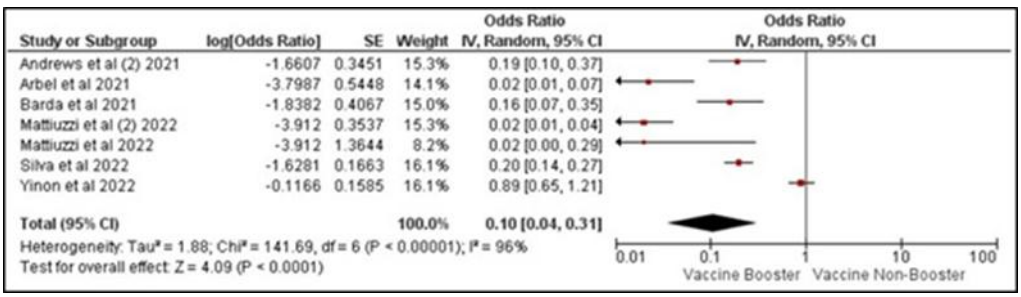

Figure 12. Forest Plot with outcome against death by BNT162b2 vaccine booster.

Figure 12 shows Vaccine Booster BNT612b2 Against Death. The urgency of vaccine to prevent death is high. The two doses of vaccine are essential, but over time the effectiveness wanes. Booster vaccine is there to help. Study showed that in people given BNT162b2 vaccine had 90%lower mortality than those who were not. The study was done on people who were 50 years old age and older, with deaths totaling 65 and 137 in both booster and non-booster participants respectively at 54 days' time (95%CI, 0.07-0.14; p<0.001)[29]. This finding is also supported by other research in individuals of 50 years old of age, which showed that vaccine effectiveness was 97.8 over 14-34 days after booster was injected (95%CI, 94.4-99.1).

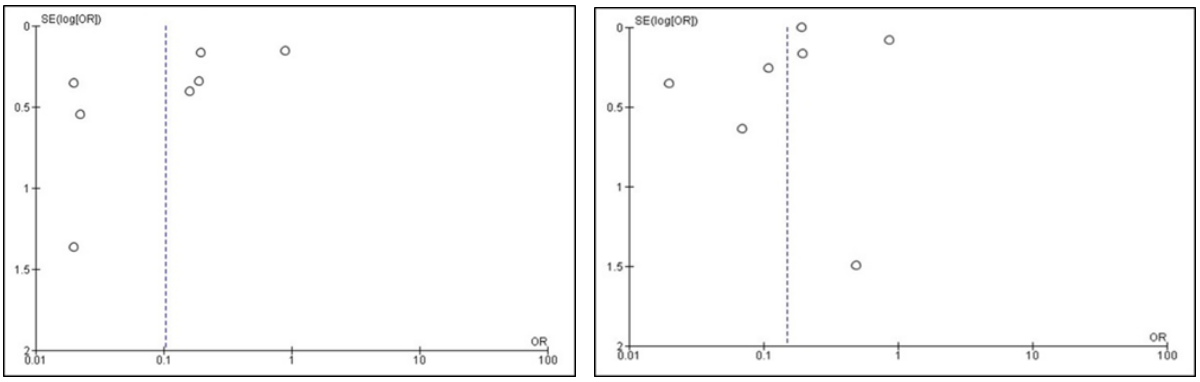

Figure 13. Funnel Plot with outcome against death by BNT162b2 vaccine booster.

Figure 13 indicates that there is no publication bias, because the structure of the image is symmetrical between right and left. Publication bias can be influenced by several factors. Here are some common ones:

Editorial Preferences: Journal editors play a crucial role in deciding which studies get published. Their preferences, biases, and interests can impact the selection process[12].

Selective Reporting: Researchers may choose not to submit or publish studies with negative findings (often called "negative studies"). They might perceive these results as less interesting or consider their research to have "failed" if the effects are not statistically significant.

Funding Influences: Financial interests, conflicts of interest, or pressure from funders can affect the decision to publish certain results. Researchers may suppress negative findings from clinical trials to maintain funding or align with specific agendas.

Publication bias distorts the overall body of evidence and can impact decision-making across various fields. It's essential to recognize and address these factors to ensure a more balanced representation of research outcomes.

DISCUSSION

The high number of infections from the coronavirus necessitates accelerated efforts in providing vaccination coverage. The waning protection from the vaccine against COVID-19 and the introduction of SARS-CoV-2 Omicron (B.1.1.529) variant have prompted efforts to scale up COVID-19 booster immunization. Booster doses are given to people who have already been vaccinated and have completed their primary period of prevention (presently first and second doses of vaccine against COVID-19 based on the type of vaccine).

Boosters can help to protect the clinically susceptible and unvaccinated by lowering infection. This study provides an analysis related to the effectiveness of the booster vaccine against many aspects that have not been carried out by other journals, namely confirmed infections, against symptomatic disease and asymptomatic disease, also severity and death[12,13].

There are 16 observational studies (Muhsen et al., 2022[14]; Spitzer et al., 2022[15]; Mattiuzzi et al., 2022[16]; Mattiuzzi et al., 2022[17]; Berec et al., 2021[18]; Bar-On et al., 2021[19]; Bar- On et al., 2021[20]; Andrews et al., 2021[21]; Abu Raddad et al., 2022[22]; Silva et al., 2022[23]; Andrews et al., 2022[24]; Barda et al., 2021[25]; Uzun et al., 2022[26]; Robles-Fontan et al., 2021[27]; Sritipsukho et al., 2021[28]; Arbel et al., 202[29]) which were analyzed qualitatively with a systematic review. The studies were carried out in nine different countries. Of the 16 studies, only 13 were analyzed quantitatively by meta-analysis.

Vaccine booster BNT162b2 Against Confirmed Infection

Our meta-analysis of six studies found booster vaccine became powerful in reducing the quantity of infections. This study showed the number of confirmed SARS COV-2 infections was decreased with the booster against the non-booster. Giving a booster of BNT162b2 vaccination can increase antibody neutralization. Increased neutralization titers will provide protection against infection. The infection rate in the booster group was lower than the non-booster group by a factor of 11.3 (95% confidence interval [CI], 10.4 to 12.3). The number of infections was lower on days 12 to 25 post-booster compared to four to six days post-booster [20]. This is also supported by a study conducted by Omer and Malani (2021) who compared the incidence of infection in the mRNA booster group, two doses of mRNA vaccine and those without vaccine[30]. In this study, bias was found, which may be caused by data sources, the presence of comorbidities, differences in behavior to seek help in the booster and non-booster groups. In addition, it could be caused by the limited sample, the low incidence of asymptomatic infection, and some patients who did not perform the PCR test.

A study conducted in Israel assessed the effectiveness of a booster dose of the BNT162b2 vaccine (Pfizer-BioNTech) against SARS-CoV-2 infection and showed a value of 96.8% for people aged 16-59 years and 93.1% for people over 60 years in week three. However, there was a decrease in vaccine effectiveness after eight weeks in the 16-59 year age group and 11 weeks in the over 60 year age group. This decline became more pronounced in the final 2-3 weeks of evaluation, with estimates of vaccine effectiveness reaching 77.6% and 61.3% for people aged 16-59 years and over 60 years, respectively. This decrease occurred at the same time as the activity of the Omicron variant increased. Despite this, vaccines continue to provide moderate to high protection against cases of abuse, including reductions in cases, hospitalizations, and deaths associated with COVID-19[31].

Vaccine Booster BNT162b2 Against Symptomatic Disease

Based on the results of meta-analysis of five studies, it was found that BNT162b2 booster was effective against symptomatic disease-related COVID-19, with a significant association ($P < 0.05$). The heterogeneity of the study was 100%, with an OR 0.22 (95% CI 0.11-0.44). The results of a study evaluating the third dose of BNT162b2 effectivity in the United States unified health order show that vaccine effectivity declines following two

doses of BNT162b2 and that obtaining dose number three gives different protection against the infection of SARS-CoV-2 COVID-19 in-patients than only receiving two doses. Given that 91% of the infections were symptomatic, the study found no significant differences in the adjusted effectiveness of three BNT162b2 dosages against symptomatic COVID-19 (90 %[95% CI 88-92])[32]. Our review also has limitations. Of the study variants, two were case-control observational studies and the other three were cohort studies.

A prospective cohort study was conducted in Hong Kong to measure the effectiveness of BNT162b2 and CoronaVac vaccines against asymptomatic and symptomatic SARS-CoV-2 omicron infections. The study involved 8636 individuals aged 5 years and older, who were enrolled from all 18 districts of Hong Kong. The primary outcomes were the incidence of SARS-CoV-2 infection and the vaccine effectiveness of BNT162b2 and CoronaVac vaccines. The study found that statistically significant protection against asymptomatic and symptomatic SARS-CoV-2 omicron infection was found only for those who received a BNT162b2 or CoronaVac booster dose, with a vaccine effectiveness of 41.4% and 32.4%, respectively. The vaccine effectiveness of BNT162b2 and CoronaVac boosters was further increased to 50.9% and 41.6% for symptomatic omicron infections. A similar pattern of vaccine effectiveness was also conferred after receipt of a BNT162b2 booster by individuals who received a CoronaVac primary vaccination series[33].

Vaccine Booster mRNA-1273 Against Symptomatic Disease

From the two studies that we reviewed to assess mRNA-1273 booster vaccine effectivity to avert the occurrence of symptomatic infection of SARS CoV-2, the results were significant with OR 0.30 (95% CI 0.10 – 0.94) and P value = 0.04. Using the random effect model (REM) analysis model, the heterogeneity of the two studies was above 50% (I2: 97%, P < 0.00001), which means that it has a high level of heterogeneity. One of the included studies, by Andrews et al. (2021) also found mRNA booster vaccines against symptomatic disease in the age group 50 years and above are effective against symptomatic disease[21]. According to research studies from Doria-Rose et al. (2021), dose number three of mRNA-1273 can increase neutralization of the Omicron titer and can substantially decrease the possibility of disease with symptoms in COVID-19 infection[32]. In this study, publication bias was found because there were differences in study designs, namely case control vs retrospective cohort studies, differences in population size, differences in age groups compared, and differences in the time span of booster vaccines after the second vaccine.

Vaccine Booster BNT612b2 Against Asymptomatic Disease

SARS-CoV-2 contaminations are frequently symptomless and can present a possibility to susceptible people, making them rare breakthrough infections with contagious potential that pose a unique issue[33]. Identifying their possibility to disseminate the agent along a period of time of direct contact with an infected person along the general communal, decreases the possibility of contagious contamination, This may take place among patients with symptomless contamination or some period of time prior to the onset of the signs, and is mostly principal among health control employees, initial staff, also further important and forefront staff[34]. The noteworthy findings were based on a three

literature reviews of BNT162b2 effectivity booster doses in order to prevent asymptomatic infection of SARS-CoV-2. This outcome is more consistent with earlier research. The Moderna mRNA and Pfizer-BioNTech vaccines against COVID-19 were around 90% successful in order to prevent asymptomatic and symptomatic contamination with SARS-CoV-2 32, according to a web of prospective cohorts between forefront staff. The Pfizer–BioNTech BNT162b2 messenger RNA vaccine has also been demonstrated to lower the incidence of asymptomatic illness and the related infectivity [33].

Vaccine Booster against Hospitalization

In terms of COVID-19-related urgent care/emergency room (UC/ER) for in-patients and visits, vaccine effectiveness (VE) increased after dose number three much more than after dose number two but decreased slowly since vaccination. In the time of mainly Omicron variant, the effectivity of vaccines against COVID-19 associated urgent care/emergency room in-patients and visits were 97% and 91%. But throughout the two months after booster dose, this dropped to 66% and 78% in the fourth month after booster dose[35]. This meta-analysis consisting of six studies of BNT162b2 booster vaccine shows that booster vaccine is effective in terms of reducing hospitalizations. The result is significant (P<0.000) and the heterogeneity of the study is 93% with an OR of 0.12 (95% CI 0.06-0.22).

Vaccine Booster BNT612b2 Against Severe Disease

Bar-On et al. (2021) compared COVID-19 confirmation rates and severe illness rates in individuals who had had a booster injection at least 12 days before (booster group) with those who had not had a booster shot (non-booster group) in the primary study. The verified rate of serious illness was reduced by a factor of 19.5 (95% CI, 12.9–29.5) at least 12 days following the booster dose. Those who had a booster (third) dose of BNT162b2 vaccination had significantly reduced rates of confirmed COVID-19 and severe illness, according to Bar-On et al. (2019) [19]. From the seven studies that we used to evaluate the effectiveness of the BNT162b2 booster vaccine to prevent severity, the results were significant with OR 0.15 (95% CI 0.07 – 0.33) and P<0.00001. The heterogeneity of the seven studies was above 50% (I2: 98%, P <0.00001), which means there was a high degree of heterogeneity.

Vaccine Booster BNT612b2 Against Death

The urgency of vaccine to prevent death is high. The two doses of vaccine are essential, but over time the effectiveness wanes . Booster vaccine is there to help. Study showed that people given BNT162b2 vaccine had 90% lower mortality than those who were not. The study was done on people who were 50 years old age and older, with total deaths of 65 and 137 in both booster and non-booster participants, respectively, at 54 days' time. (95%CI, 0.07-0.14; p<0.001)[29]. This finding is also supported by other research on individuals of 50 years old of age, which showed that vaccine effectiveness was 97.8 over 14-34 days after booster injection (95%CI, 94.4-99.1)[24].

STRENGTH AND LIMITATION

There are numerous limitations in this research. First, there are limitations in study period. Second, there may be unmeasured confounding factors such as sociodemographic factors, behavioral factors, and testing rate. Third, exploring

potential adverse effect may be concerned as limitations. Fourth, the under-reporting of patients with confirmed COVID-19 infection (whether they had a booster vaccination or not), especially when the health system is occupied, as well as the accessibility and capacity to examine and evaluate COVID-19 incidence rates for different outcomes. Fifth, there are significant population differences included in various studies regarding number of cases, gender, ethnicity, age, geographic area, level of vaccine population coverage, etc. Sixth, there were limitations in the amount of issued study literature researches. Seventh, there were limitations in the time of investigation following the vaccine booster doses. Eighth, the included studies used a variety of methods.

We used journals with four different methods (cohort, cross-sectional, retrospective cohort, case control) which guarantees the carefulness of the results interpretation. Ninth, serious occasions and more possible to have medical awareness and therefore be recorded and caught, which is the major key for monitoring severe disease and vaccine efficacy. Lastly, there are analysis limitations because of numerous research biases, similar study bias, and diversity. We performed a sub-population analysis to perceive the roots of diversity. The study described several features of the study as well as confirmed infection, severe illness, hospitalization, and death. To prevent language bias, we only used articles in English. To prevent publication influences, we looked for numerous sites and obtained data from credible sources to obtain issued works. Nevertheless, diversity and bias are unpreventable in systematic reviews and meta-analytical research. Thus, all limitations have to be reviewed during interpretation of the results.

CONCLUSIONS

From 16 observational studies conducted by systematic reviews, it was concluded that the booster vaccine group had lower rates of confirmed infections and lower severity of illness related to hospitalization and mortality. Then from the 16 observational studies that were carried out, a systematic review was carried out, followed by a meta-analysis of 13 observational studies.

From the outcomes of the meta-analysis, it was found that the booster vaccine is effective in reducing the severity of disease and preventing death in patients infected with COVID-19 with a protective Odds Ratio (OR) value < 1. The OR value of the BNT162b2 booster vaccine effectivity against confirmed infections is OR 0, 16, BNT162b2 booster vaccine against symptomatic disease is 0.22, mRNA-1273 booster vaccine against symptomatic disease is 0.30, BNT162b2 booster vaccine against asymptomatic disease is 0.72, BNT162b2 booster vaccine hospitalization is 0.12, booster vaccine BNT162b2 against severe disease is 0.15, and booster vaccine BNT162b2 against death is 0.10 This will speed up the handling of the COVID-19 disease by knowing the various conditions of the disease Fast handling of COVID-19 in accordance with the state of the disease will reduce deaths.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

MK contributed to manuscript writing and literature collection; MGP contributed to data analysis and manuscript writing; ZNR contributed to statistical analysis and grammatical check; PPS contributed to methodology; RAR contributed to literature collection; RDA contributed to investigation; AHH contributed to methodology; BU contributed to manuscript writing and as supervisor; SF contributed to manuscript writing and as supervisor.

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Efficacy of Shampoo Made from Bangle Rhizome Extract (*Zingiber montanum*) Against Head Lice (*Pediculus humanus capitis*)

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Abstract

Various head lice treatments with different mechanisms of action have been developed and explored over the years, with limited reports on systematic assessments of their efficacy and safety. The head lice shampoo commercial is 1% permethrin-based. Side effects of using permethrin-based shampoos include skin irritation and high resistance. The research conducted aims to present strong evidence that the use of shampoo made from bangle rhizome extract against head lice mortality is safer to use. The study used 240 head lice taken from elementary school students (8-12 years) in a school in Bekasi. The study group was divided into 6 groups: negative control group, positive control group (using permethrin-based anti-lice shampoo), shampoo treatment group made from bangle rhizome extract with dose of 0.5%, 1%, 2% and 4%. In addition to calculating the number of head lice deaths per group, the time of death was also calculated to determine LC_{50} and LC_{90}. The results showed a highly significant difference between the number of head lice deaths in the control group and the treatment group with shampoo made from bangle rhizome extract ($p<0.01$). Likewise, there was a highly significant difference for the time of death of head lice in the control group and the treatment group using shampoo made from bangle rhizome extract. Statistical analysis showed LC_{50} is 1% and LC_{90} is 3%. The effective dose of shampoo made from bangle rhizome extract is 4%, which can kill 100% of head lice within 27.5 minutes.

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INTRODUCTION

Human head lice (*Pediculus humanus capitis*) infestation is a global public health problem that affects people from all socioeconomic backgrounds.[1,2] Although there is a lack of reliable data, estimates of the prevalence of head lice infestation in school-aged children range from 5% in Europe to 33% in Central and South America.[3] Head lice infestation affects people regardless of ethnicity and age, although it is more common in children aged 7-14 years, females and vulnerable populations living in crowded environments.[1]

Head lice cause considerable discomfort, and intensive itching that can lead to sleep deprivation and excoriation-although rare, wounds on skin infected with resistant pathogenic bacteria can lead to secondary skin infections and lymphadenopathy.[4,5] In addition, affected children and their parents often suffer from social stigma, embarrassment, and low self-esteem.[6,7] Data on the spread of head lice in Indonesia are limited. However, research conducted by Karimah et al.[8] stated that the prevalence of Pediculosis capitis out of 123 research subjects was 55.3%.[8] Another study conducted by Wahdini et al.[9] in a boarding school in Bogor found that the prevalence of *Pediculosis capitis* was 88.4%.[9] While Fauzan and Nita[10] stated that the prevalence of Pediculosis capitis was more in girls than boys (65.1%).[10]

The head lice (*Pediculus humanus capitis*) is wingless, 2 mm to 4 mm long (at maturity), six-legged, and is a blood-sucking insect that lives on the human scalp.[11] Infested children usually have fewer than 20 adult lice at any one time, which live 3 to 4 weeks if untreated.[12-14] Head lice live close to the scalp surface which provides food, warmth, shelter and moisture.[12,14] Head lice feed every 3 to 6 hours by sucking blood and injecting saliva simultaneously. After mating, adult female lice can produce 5 or 6 eggs per day for 30 days, each attached to a hair shaft near the scalp.[13,14]

There are various interventions available for the management of head lice.[1] Mainstream therapies have largely relied on insecticide-based approaches for decades. However, accumulating evidence with resistance to front-line insecticide treatments such as pyrethrins, permethrin, and malathion has led to increasing initiatives to develop newer and more effective treatments to safely treat this condition.[1] Over the past decades, alternative candidates have been introduced to the market, including ivermectin,[15-17] occlusive agents (e.g., benzyl alcohol, isopropyl myristate, and dimethicone),[4,18,19] and herbal products,[20] and essential oils.[20-22]

Although drugs with new modes of action have the potential to address the rapidly growing resistance problem, in the absence of strong comparative evidence, the relative efficacy and safety of newer agents and how they compare to insecticide treatment remains unclear. As a result, the government is dedicating relatively large resources to develop new products and devise strategies to control and prevent head lice through the utilization of medicinal plants available nearby. This means that, with all the limitations of using pediculicides that have been circulated and used, which can cause resistance in head lice, the government through the Ministry of Health and the Ministry of Education encourages scientists to further develop the potential of medicinal plants that are effective in killing head lice without causing resistance, as well as being safer to use.

Bangle (*Zingiber montanum*) is a type of medicinal plant that has begun to be widely researched as a natural

ingredient to treat head lice. This is because bangle rhizome extract contains chemical compounds that can be used as anti-head lice. Padmasari et al.[23] stated that the results of phytochemical screening on 70% ethanol extract of bangle rhizome (*Zingiber purpureum* Roxb.) are saponins, flavonoids, triterpenoids, steroids, essential oils, alkaloids, tannins and glycosides.[23] The research conducted aims to make shampoo made from bangle rhizome extract with graded doses that effectively work as pediculicides for head lice (*Pediculus humanus capitis*).

MATERIALS AND METHODS

Materials

The tools used are measuring cup, beaker, Erlenmeyer, test tube, stirring rod, digital analytical balance, filter paper, petri dish, rotary evaporator, oven, blender, shampoo container, pH meter, viscometer, hot plate, and maceration container (Pyrex USA). The materials needed are bangle rhizomes and 70% ethanol. For the manufacture of shampoo, sodium lauryl sulfate, hydroxypropyl methylcellulose (HPMC), methyl paraben (Ginhong Mixer, China), distilled water, and 1% permethrin are needed.

Methods

Sample Preparation

Bangle rhizomes were purchased from the Kopro traditional market Tanjung Duren Jakarta Barat. The collected bangle rhizomes were washed, sliced thinly, then dried by aerating. The dried bangle rhizomes were blended until bangle rhizome powder was obtained. Sample preparation and research were carried out at the Research Laboratory of the Faculty of Medicine and Health Sciences, Krida Wacana Christian University Jakarta.

Head Lice Preparation

Human head lice or *Pediculus humanus capitis* were obtained from girls aged 8-12 years in Cirewed Village, Cikupa, Tangerang, Banten who were infested with head lice with the criteria of not having received therapy except with a special comb or comb. Sampling of lice from the respondent's hair involved using a special comb, then the lice along with the hair to which they were attached were placed in a bottle whose lid has been perforated to allow oxygen to enter. This was with the aim that the body of the louse remained normal when given treatment.

Preparation of 70% Ethanol Extract of Bangle Rhizome

A total of 200 g of bangle rhizome powder was weighed, then filtered. The dregs obtained were macerated with 500 mL of 70% ethanol at room temperature for 3-4 days, then filtered and the filtrate was collected. The filtrate was concentrated with a rotary evaporator at 50^{o}C to obtain an extract that still contained a small volume of solvent. The evaporation of the extraction solvent was continued using an oven at 40^{o}C until a thick bangle extract was obtained.

Shampoo Preparation

The shampoo formula consists of HPMC (Hydroxy Propyl Methyl Cellulose), sodium lauryl sulfate, methyl paraben and distilled water. The shampoo added bangle rhizome extract with concentrations of 0.5%, 1%, 2% and 4%. The shampoo was prepared by making a mixture of HPMC (1%) which was added little by little into hot distilled water as much as 20 mL while stirring (mixture 1). Methyl paraben was dissolved using ethanol until dissolved (mixture 2). Then 50 mL of distilled water was put in a 1 L beaker and heated on a hot

plate at 60°C, then sodium lauryl sulfate was added and stirred until homogeneous. After ii was homogeneous, mixture 1 and mixture 2 were added and stirred until thickened. Then bangle rhizome extract was added according to the predetermined concentration, along with distilled water as much as 100 mL.[24] The finished shampoo was put into each bottle according to the predetermined concentration.

Research Stages

The research group was divided into six, namely the negative control group (given baby shampoo), the positive control group (given 1% permethrin-based anti-lice shampoo), the treatment group of bangle rhizome extract concentrations of 0.5%, 1%, 2% and 4%. Each group was given 10 lice, with four replications. After the head lice were collected, testing was done immediately because head lice can only survive for 24 hours after leaving the scalp.[25] Treatments were given by placing 10 lice on a Petri dish then given shampoo, and repeated up to four times for each group. Observations were made by counting the number and time of death of head lice. The indicator of a dead louse is that the louse does not move when touched gently with tweezers, or is a rigid body condition with an irregular leg position, not moving, and not responding to stimuli when touched.[26]

Data Analysis

The data obtained were then analyzed using the one-way ANOVA test and the least significant difference (LSD) test to determine the group that most effectively killed head lice in less than two hours (120 minutes). Probit test was conducted to determine the effective dose and time of death of head lice given shampoo made from bangle extract with graded doses. Data were processed using SPSS software version 27.0.

RESULTS AND DISCUSSION

The head lice used in the study were taken from the heads of elementary school children aged 8-12 years. This is because this age range is more exposed to head lice.[27]

The average percentage of head lice killed by shampoo treatments made from 1% permethrin and made from bangle rhizome extract at graded doses can be seen in Figure 1.

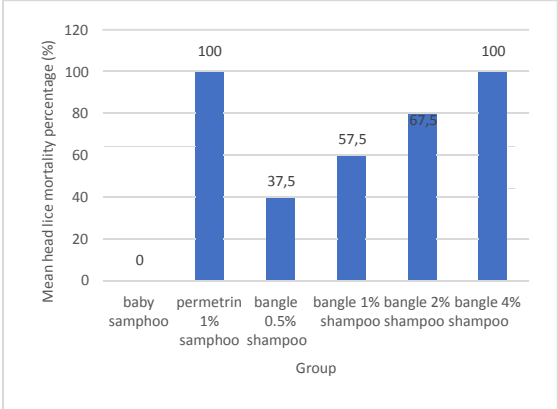

Figure 1. Mean percentage of head lice mortality in the control and treatment groups.

Based on the one-way ANOVA test in Table 1, it was found that there was a significant difference in the mean percentage of head lice mortality between the negative control group (baby shampoo), the positive control group (permethrin 1 shampoo), and the treatment group with shampoo made from bangle rhizome extract with graded doses ($p<0.01$). The test continued with the least significant difference (LSD) as shown in Table 2, the results of which stated that the negative control group was significantly different from the positive control group and the treatment group with shampoo made from bangle rhizome extract in graded doses ($p<0.01$), while the positive control group was not significantly different from the shampoo treatment group made from 4% dose of bangle

Table 1. One-way ANOVA Test of Mean Percentage of Head Lice Mortality in Control and Treatment Groups.

			Sum of Squares	df	Mean Square	F	Sig
Between Groups	(Combined)		294.708	5	58.942	103.507**	0.000
	Linear Term	Contrast	102.004	1	102.004	179.128	0.000
		Deviation	192.705	4	48.176	84.602	0.000
Within Groups			10.250	18	0.569		
Total			304.958	23			

** Significantly different (p<0.01)

rhizome extract. This means that the positive control group (permethrin 1%) and the treatment group with shampoo made from 4% dose of bangle rhizome extract showed an effective function to kill head lice (100%).

Table 2. Least significant difference test on head lice mortality rate

I Group	J Group	Mean Difference (I-J)
Baby shampoo	Permethrin 1%	-10.000*
	Bangle 0.5%	-3.750*
	Bangle 1%	-5.750*
	Bangle 2%	-6.750*
	Bangle 4%	-10.000*
Permethrin 1%	Baby shampoo	10.000*
	Bangle 0.5%	6.250*
	Bangle 1%	4.250*
	Bangle 2%	3.250*
	Bangle 4%	.000
Bangle 0.5%	Baby shampoo	3.750*
	Permethrin 1%	-6.250*
	Bangle 1%	-2.000*
	Bangle 2%	-3.000*
	Bangle 4%	-6.250*
Bangle 1%	Baby shampoo	5.750*
	Permethrin 1%	-4.250*
	Bangle 0.5%	2.000*
	Bangle 2%	-1.000*
	Bangle 4%	-4.250*
Bangle 2%	Baby shampoo	6.750*
	Permethrin 1%	-3.250*
	Bangle 0.5%	3.000*
	Bangle 1%	1.000*
	Bangle 4%	-3.250*
Bangle 4%	Baby shampoo	10.000*
	Permethrin 1%	.000
	Bangle 0.5%	6.250*
	Bangle 1%	4.250*
	Bangle 2%	3.250*

*. The mean difference is significant at the 0.05 level.

The average time of death of head lice treated with 1% permethrin-based shampoo and bangle rhizome extract at graded doses can be seen in Figure 2.

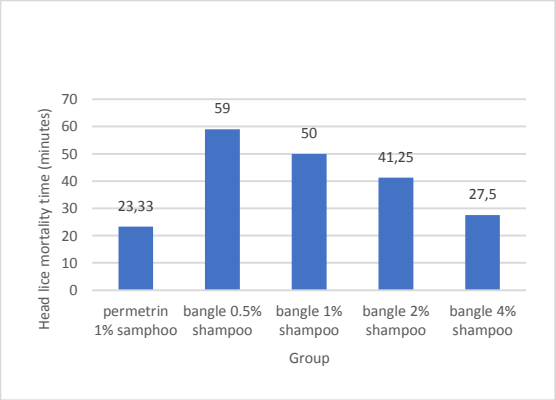

Figure 2. Mean time to death of head lice in control and treatment groups.

Based on the one-way ANOVA test in Table 3, it was found that there was a significant difference in the meantime of head lice death between the negative control group (baby shampoo), the positive control group (permethrin 1% shampoo), and the treatment group with shampoo made from bangle rhizome extract with graded doses ($p<0.01$). The test continued with the least significant difference (LSD) and, as seen in Table 4, the results stated that the negative control group was significantly different from the positive control group and the treatment group with shampoo made from bangle rhizome extract in graded doses ($p<0.01$), while the positive control group was not significantly different from the treatment group with shampoo made from 4% dose of bangle rhizome extract. This means that the positive control group (permethrin 1%) and the treatment group with shampoo made from 4% dose of bangle rhizome extract had an effective time to kill head lice (within 23 minutes and 27 minutes, respectively).

Figure 1 shows that 1% permethrin-based shampoo (positive control) was the most effective pediculicide with 100% head lice mortality at 23 minutes, followed by 4% bangle rhizome extract-based shampoo which showed 100% head lice mortality at 27 minutes. As for the shampoo made from 2% bangle rhizome extract (67.5% head lice died within 41 minutes), shampoo made from 1% rhizome extract (57.5% head lice died within 50 minutes), and shampoo made from 0.5% bangle rhizome extract (37.5% head lice died within 59 minutes). Meanwhile, the negative control group using baby shampoo showed no head lice eradication effect, with 100% of head lice surviving throughout the study.

Permethrin used as a positive control in the study is a type of synthetic pyrethroid marketed in shampoos at a concentration of 1%. It has been used to treat head lice for more than 50 years, but has recently received greater attention due to its toxic side effects and resistance.[28-30]

Resistance to the use of pyrethroid insecticides on head lice has been widely studied, including research conducted by Brownell et al.[31] which stated that there was a 40% mutation of head lice in Thailand.[31] Research by Roca Acevedo et al.[32] in Chile found 50% of head lice having mutations in the T9171 allele.[32]

The effectiveness of 1% permethrin-based shampoo as a pediculicide is due to the fact that permethrin is a synthetic neurotoxic pyrethroid that acts on the nervous system used in the treatment of pediculosis.[33,34] Its mechanism of action is to disrupt sodium channels causing depolarization, thus causing respiratory paralysis in head lice.[34] Permethrin also slows the closure of Na ion channels or canals, causing neuronal dysfunction. This causes respiratory paralysis in head lice.[30,35]

Meurer-Grimes et al.[36] gave a different statement about how permethrin works on the insect body.[36] When insects are exposed to permethrin compounds, it interacts with voltage gated sodium channels that cause sodium channels to not close so that the repolarization process occurs.[37,38] Furthermore, it will cause interference in the transmission of nerve signals and the result is that the insect has difficulty controlling its muscle movements (convulsions). Damage that interferes with muscle control can also cause paralysis and loss of ability to perform vital functions leading to insect death.[30]

The death that occurred in the head lice of the treatment group with shampoo made from bangle extract with graded doses was due to the flavonoid and saponin compounds contained therein. This is in line with the results of research

Table 3. One-way ANOVA Test of Mean Time to Death of Head Lice in Control and Treatment Groups.

			Sum of Squares	df	Mean Square	F	Sig
Between Groups	(Combined)		3589.583	4	897.396	7.561**	0.000
	Linear Term	Unweighted	31.859	1	31.859	0.268	0.000
		Weighted	229.066	1	229.066	1.930	0.000
		Deviation	3360.517	3	1120.172	9.437	
Within Groups			1780.417	15	118.694		
Total			5370.000	19			

**** Significantly different (p<0.01)**

conducted by Putri et al.[39] which states that overcoming head lice with insecticides made from medicinal plants containing flavonoid compounds can interfere with digestion. Apart from flavonoid compounds, saponin compounds can also cause head lice death. Saponins can inhibit growth, damage cell membranes, and disrupt metabolism in the body of head lice. Saponins enter the insect body by inhibiting protease enzymes, which results in decreased nutrient intake and forms protein complexes, and inhibits growth.[39]

Table 4. Least significant difference test on the meantime of head lice death

I Group	J Group	Mean Difference (I-J)
Permethrin 1%	Bangle 0.5%	-35.667*
	Bangle 1%	-26.667*
	Bangle 2%	-17.917*
	Bangle 4%	-4.167
Bangle 0.5%	Permethrin 1%	35.667*
	Bangle 1%	9.000*
	Bangle 2%	17.750*
	Bangle 4%	31.500*
Bangle 1%	Permethrin 1%	26.667*
	Bangle 0.5%	-9.000*
	Bangle 2%	8.750*
	Bangle 4%	22.500*
Bangle 2%	Permethrin 1%	17.917*
	Bangle 0.5%	-17.750*
	Bangle 1%	-8.750*
	Bangle 4%	13.750*
Bangle 4%	Permethrin 1%	4.167
	Bangle 0.5%	-31.500*
	Bangle 1%	-22.500*
	Bangle 2%	-13.750*

*. The mean difference is significant at the 0.05 level.

Based on the Probit test, the use of shampoo made from bangle rhizome extract at graded doses of up to 4%, obtained an LC_{50} value of 1%, and LC_{90} of 3%.

Table 5. Probit Test of Bangle Dosage on Head Lice Mortality

	Probability	Estimate (95% Confidence Limits)
Probit	.500	1.000
	.900	3.000
	.990	4.000

Therefore, herbal shampoo made from bangle rhizome extract with graded concentrations up to 4% is expected to be used as an alternative product to control head lice (pediculicide), because it can kill 100% of head lice.

STRENGTH AND LIMITATION

Bangle rhizomes are easily and widely found in Indonesia, including Jakarta. The limitation of this study is the difficulty of obtaining head lice in large quantities.

CONCLUSIONS

The group of head lice treated with 1% permethrin-based commercial shampoo experienced 100% mortality within 23.33 minutes, which was not significantly different from the group of head lice treated with 4% bangle rhizome extract-based shampoo (100% mortality within 27.5 minutes). The effective dose of bangle rhizome extract that can kill 50% (LC_{50}) of head lice is 1%, and that can kill 90% (LC_{90}) of head lice is 3%.

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ETCHICAL CLEARANCE

The research protocol was approved by the Health Ethics Committee of the Faculty of Medicine and Health Sciences, Krida Wacana Christian University Jakarta, NoSLKE: 1577/SLKE-IM/UKKW/FKIK/KE/VIII/2023

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CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

AUTHOR CONTRIBUTION

Conceived and designed the experiments: RPS. Peformed the experiments: RPS, AWS. Analyzed the data: MPS. Contributed reagents/materials/analysis tools: AWS. Wrote the paper: RPS, MPS.

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3. Clancy JL, Patel HR, Hussein SM, Tonge PD, Cloonan N, Corso AJ, et al. Small RNA changes enroute to distinct cellular states of induced pluripotency. Nature communications.2014; 5:5522. Epub 2014/12/11.

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