

Correlation Between the Bacterial and Fungal Profiles from the Clinical Specimens with the CD4 Counts and the NLR Values of HIV/AIDS Patients at Tertiary Referral Hospital in Indonesia

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Original Article

Correlation between the Bacterial and Fungal Profiles from the Clinical Specimens with the CD4 Counts and the NLR Values of HIV/AIDS Patients at Tertiary Referral Hospital in Indonesia

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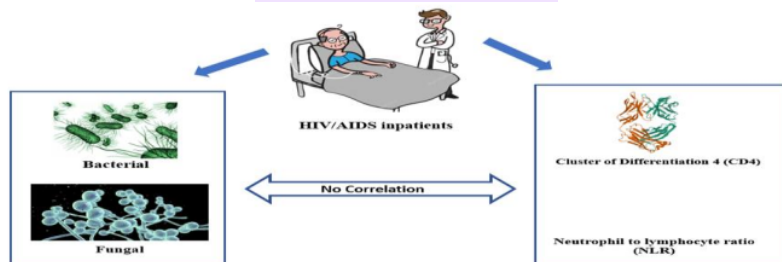
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ABSTRACT

Human immunodeficiency virus (HIV) poses a significant problem for the global public health with high morbidity and mortality rates. HIV/AIDS cases in Indonesia, the country with the fifth highest risk of HIV/AIDS in Asia, continue to increase. A decrease in CD4 levels indicates an increase in the opportunistic infection rates of HIV patients. In addition, Neutrophil to Lymphocyte Ratio (NLR) can serve as the basis for bacterial infection, infection rate, and antibiotic therapy. This observational analytic study applies a retrospective cross-sectional design and aims to observe the correlation between bacterial and fungal profiles of clinical specimens and the CD4 cell counts and the NLR values of HIV/AIDS inpatients at Dr. Soetomo Tertiary Referral Hospital from September 2021 to August 2022. A total of 192 bacterial and fungal identification test results from 76 patients were collected, consisting of 83.3% bacterial culture tests, consisting of 52.5% negative cultures, 12% gene expert TB tests, and 4.7% fungal culture tests. *Pseudomonas aeruginosa* and *Escherichia coli* ESBL were the highest numbers of Gram-negative bacteria found in this study, amounting to 51.33%, followed by *Streptococcus viridans*, the highest number of Gram-positive bacteria found in this study, amounting to 48.67%. A total of 34.2% commensal bacteria and 21.05% Multi-Drug Resistant Organism (MDRO) bacteria were also found in this study. The gene expert TB tests resulted in 73.9% undetected *M. tuberculosis*, while the fungal culture tests resulted in 88.9% *Candida albicans*. Furthermore, there was no correlation between the bacterial and fungal profiles from clinical specimens with CD4 counts or NLR values in HIV/AIDS-infected patients.

GRAPHICAL ABSTRACT



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Introduction

Human immunodeficiency virus (HIV) poses a significant problem for global public health [1] and is one of the most life-threatening infectious diseases [2] with high morbidity and mortality rates [3]. Data shows that HIV/AIDS cases in Indonesia, the country with the fifth highest risk of HIV and AIDS in Asia, continue to increase annually, although it tends to fluctuate [4, 5]. In addition, this disease has a high mortality rate in Indonesia [3].

Although HIV is the initial cause of AIDS, most deaths occur in HIV-infected patients with complications of Opportunistic Infections (OI). OI quickly attacks patients with low cellular and humoral immune defense conditions [6]. The World Health Organization (WHO) states that patients with stage III and IV HIV have a 9.4 and 22.6 times higher risk of developing OI than those with stage I of HIV. HIV-infected patients with a T-CD4 cell count of fewer than 200 cells per mm^3 were found to have a 4.9 times higher risk of developing OI than HIV-infected patients with a CD4-T cell count of more than 350 cells per mm^3 [6].

Opportunistic infections arise due to decreased immunity and can attack several organs, such as the respiratory tract, digestive tract, neurological organs, skin, and so forth [7, 8]. Such infections are a significant cause of hospitalization, morbidity, and mortality (accounting for 94.1% of all deaths) in patients with HIV/AIDS [9], especially those in low- and middle-income countries [10-12].

Severe bacterial infections other than tuberculosis should be recognized as HIV-associated opportunistic diseases [13] and the most common complications [1], as well as a primary cause of morbidity and death in patients with HIV/AIDS [14]. Meanwhile, fungi are the main contributors to OI that affect patients with HIV/AIDS and contribute significantly to OI in patients with late-stage HIV infection [15].

CD4 cell count is the most appropriate choice of the early indicators of the severity of HIV infection. A decrease in the CD4 cell count indicates an increase in the OI rate in HIV-infected patients [16, 17]. The neutrophil-to-

lymphocyte ratio (NLR) can serve as a basis for bacterial infection, infection rate, and antibiotic therapy [18], as well as an inflammation marker for diagnosing HIV and a warning for HIV severity [19, 20]. NLR is obtained from the absolute neutrophil count divided by the absolute lymphocyte count through a laboratory test, which aims to determine the occurrence of an inflammatory process [21]. The higher the NLR value, the lower the CD4 cell count, indicating that the relevant patients with HIV/AIDS are susceptible to other infections. Therefore, NLR is used as a warning of the infection severity [22]. This study is the first to observe the correlation between the bacterial and fungal profiles with the CD4 counts and the NLR values of HIV/AIDS-infected patients.

Materials and Methods

This analytic observational study applied a retrospective cross-sectional approach. The study population included medical record data of HIV/AIDS-infected patients aged ≥ 18 years old treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital from September 2021 to August 2022.

The inclusion criteria in this study included the HIV/AIDS inpatients treated in the special inpatient ward for HIV/AIDS isolation who sent their results of bacterial or fungal identification tests of all specimens to the clinical microbiology laboratory with complete CD4 and NLR data. Ethical clearance for this study has been issued by the Health Research Ethics Committee of Dr. Soetomo Tertiary Referral Hospital, Surabaya, based on a certificate of ethical clearance No. 1060/LOE/301.4.2/X/2022.

Descriptive analysis is displayed as tables containing frequency and percentage data. Likewise, the analysis was performed using the SPSS software with a 95% confidence interval. Data were analyzed univariately to obtain descriptive results regarding the patients' characteristics, including age, sex, types of OI, bacterial and fungal profiles, and CD4 profiles. The correlation test was utilized to determine the correlation between the CD4 cell counts and the

bacterial and fungal profiles of HIV/AIDS patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Regional Public Hospital and the correlation between NLR value and the bacterial and fungal profiles of HIV/AIDS patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital.

Results and Discussion

Resistance rate of bacteremia isolates due to CRAB

The total number of HIV-infected patients treated in the special inpatient ward for HIV/AIDS isolation who sent their results of bacterial and

fungal identification tests in the Microbiology Laboratory at Dr. Soetomo Regional Public Hospital during the study period from September 2021 to August 2022 amounted to 206 patients, and those who had complete CD4 and NLR data amounted to 76 patients. A total of 192 microbiological examination results for the identification of bacteria and fungi were obtained, which were associated with the patients' CD4 counts and NLR values.

Characteristics of the HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation

Table 1: Characteristics of the HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

Characteristics	Number	Percentage
Sex		
Male	59	77.6
Female	17	22.4
Age categories		
15-19 years old	0	0
20-24 years old	10	13.6
25-49 years old	55	76.3
≥ 50 years old	11	10.2
HIV infection stages		
Stage 1 (CD4 counts of > 500)	3	3.26
Stage 2 (CD4 counts of 200-499)	10	10.87
Stage 3 (CD4 counts < 200)	79	85.87
ARV History		
Treatment-naive HIV/AIDS	34	44.7
HIV/AIDS on ARV	37	48.7
Drug-withdrawal HIV/AIDS	5	6.6
NLR Values		
CD4 counts of > 500	7.6 + 0.73	
CD4 counts of 200-499	11 + 5.68	
CD4 counts of 100-199	7 + 4.54	
CD4 counts of 50-99	13.6 + 11.07	
CD4 counts of < 50	11.02 + 9.60	
Number of opportunistic infections		
No opportunistic infection	22	28.9
One type of opportunistic infection	30	39.5
Two types of opportunistic infection	21	27.6
Three types of opportunistic infection	2	2.6
Four types of opportunistic infection	1	1.3
Types of opportunistic infection		
Pulmonary TB	18	23.7
Extrapulmonary TB	1	1.3
Encephalitis	3	3.9

	PCP	10	13.2
	Toxoplasmosis	14	18.4
	Diarrhea	12	15.8
	Hepatitis A	1	1.3
	Hepatitis B	5	6.6
	Hepatitis C	2	2.6
	Oral candidiasis	16	21.1
	ISK	11	14.5
	Pneumonia	22	28.9
Administration of antibiotic therapy			
	Receive antibiotic therapy	159	82.8
	Do not receive antibiotic therapy	331	17.2
Albumin, hematological, & electrolyte abnormalities			
	Hypoalbuminemia	43	56.6
	Anemia	37	48.7
	Leukopenia	3	3.9
	Pancytopenia	3	3.9
	Thrombocytopenia	1	1.3
	Hyponatremia	34	44.7
	Hypokalemia	20	26.3
	Hyperkalemia	4	5.3
	Hypernatremia	3	3.9

Table 2 presents that the lower the CD4 counts, the more OI the patient has. A study at Bandar Lampung Hospital, Indonesia, indicated a strong correlation between the CD4 cell counts of patients with HIV/AIDS and the incidence of OI. CD4 counts can serve as a good marker for assessing the HIV development and the OI possibility [23-25]. Meanwhile, a study on HIV/AIDS patients who had been taking ARVs for six months at the VCT outpatient department of Surakarta Hospital, Indonesia, stated that the OI incidence was affected by CD4 cell counts, not by viral load and NLR values [26, 27].

A study conducted by Subhas B. *et al.*, in 2015-2018 in India, found that there were hematological abnormalities at all stages in HIV AIDS patients. These disorders may include anemia, leukopenia, lymphocytopenia, and thrombocytopenia [28] as presented in Table 3. The hematological profile of HIV-infected patients appears to reflect the degree of viral replication, with severe abnormalities observed in patients with reduced CD4 cell counts and high viral loads [29]. It is in accordance with Table 3, which shows that the lower the CD4 count, the

higher the incidence of hematological abnormalities.

Cytopenia develops due to various mechanisms. HIV infection causes increased expression of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α), transforming growth factor-beta (TGF- β), and interleukin-1 (IL-1), which result in myelosuppression and changes in the bone marrow microenvironment. Immune-mediated destruction of blood cells may also occur. Opportunistic infections, malignancies, and HIV treatment can further contribute to developing cytopenias [30, 31].

Several studies have revealed anemia as the most common cytopenia in HIV-infected individuals, as seen in Table 3. The Brazilian study further determined that anemia in HIV patients was independently associated with death, making hemoglobin a valuable prognostic biomarker [32, 29].

Several factors contribute to anemia in HIV-infected patients. Several studies have shown that the pathophysiology of anemia in HIV is influential. Another factor that contributes to anemia in HIV-infected individuals is CD4 count. A study conducted by Sang Bagus *et al.* (2018), in

Bali, found a significant correlation between Hb levels and CD4 counts. Anemia can serve as a marker for decreased CD4 counts, so a simple complete blood count can support the diagnosis of a decreased CD4 count [17]. It is in accordance with the results of this study presented in Table 3, which demonstrates that incidence of anemia increases with lower CD4 count.

Table 2: Distribution of opportunistic infections by CD4 counts

Opportunistic infections	CD4 counts of > 500 $\Sigma = 2$ n (%)	CD4 counts of 200-499 $\Sigma = 5$ n (%)	CD4 counts of 100-199 $\Sigma = 10$ n (%)	CD4 counts of 50-199 $\Sigma = 17$ n (%)	CD4 counts of < 50 $\Sigma = 42$ n (%)
Pulmonary TB					
Yes ($\Sigma = 18$)	0 (0)	0 (0)	4 (40)	6 (35.3)	8 (19)
No ($\Sigma = 58$)	2 (100)	5 (100)	6 (60)	11 (64.7)	34 (81)
Extrapulmonary TB					
Yes ($\Sigma = 1$)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.4)
No ($\Sigma = 75$)	2 (100)	5 (100)	10 (100)	17 (99.4)	41 (97.6)
Encephalitis					
Yes ($\Sigma = 3$)	0 (0)	0 (0)	0 (0)	1 (5.9)	2 (4.8)
No ($\Sigma = 73$)	2 (100)	5 (100)	10 (100)	16 (94.1)	40 (95.2)
PCP					
Yes ($\Sigma = 10$)	0 (0)	0 (0)	3 (30)	1 (5.9)	6 (14.3)
No ($\Sigma = 66$)	2 (100)	5 (100)	7 (70)	16 (94.1)	36 (85.7)
Toxoplasmosis					
Yes ($\Sigma = 14$)	0 (0)	0 (0)	2 (20)	4 (23.5)	8 (19)
No ($\Sigma = 62$)	2 (100)	5 (100)	8 (80)	13 (76.5)	34 (81)
Diarrhea					
Yes ($\Sigma = 12$)	0 (0)	0 (0)	5 (50)	2 (11.8)	5 (11.9)
No ($\Sigma = 64$)	2 (100)	5 (100)	5 (50)	15 (88.2)	37 (88.1)
Hepatitis A					
Yes ($\Sigma = 1$)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)
No ($\Sigma = 75$)	2 (100)	5 (100)	9 (90)	17 (100)	42 (100)
Hepatitis B					
Yes ($\Sigma = 5$)	0 (0)	0 (0)	0 (0)	3 (17.6)	2 (4.8)
No ($\Sigma = 71$)	2 (100)	5 (100)	10 (100)	14 (82.4)	40 (95.2)
Hepatitis C					
Yes ($\Sigma = 2$)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.8)
No ($\Sigma = 74$)	2 (100)	5 (100)	10 (100)	17 (100)	40 (95.2)
Oral candidiasis					
Yes ($\Sigma = 16$)	0 (0)	0 (0)	3 (30)	1 (5.9)	12 (28.6)
No ($\Sigma = 60$)	2 (100)	5 (100)	7 (70)	16 (94.1)	30 (71.4)
ISK					
Yes ($\Sigma = 11$)	1 (50)	1 (20)	1 (10)	2 (11.8)	6 (14.3)
No ($\Sigma = 65$)	1 (50)	4 (80)	9 (90)	15 (88.2)	36 (85.7)
Pneumonia					
Yes ($\Sigma = 22$)	0 (0)	1 (20)	1 (10)	3 (12.6)	17 (40.5)
No ($\Sigma = 58$)	2 (100)	4 (80)	9 (90)	14 (82.4)	25 (59.5)

Table 3: Albumin, hematological, and electrolyte abnormalities in HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital based on CD4 counts

Albumin, hematological, and electrolyte abnormalities	CD4 counts of >500 Σ = 2 n (%)	CD4 counts of 200-499 Σ = 5 n (%)	CD4 counts of 100-199 Σ = 10 n (%)	CD4 counts of 50-99 Σ = 17 n (%)	CD4 counts of <50 Σ = 42 n (%)
Hypoalbuminemia					
Yes (Σ = 43)	2 (100)	2 (40)	4 (40)	10 (58.8)	25 (59.5)
No (Σ = 33)	0 (0)	3 (60)	6 (60)	7 (41.2)	17 (40.5)
Anemia					
Yes (Σ = 37)	1 (50)	3 (60)	5 (50)	12 (70.6)	16 (38.1)
No (Σ = 39)	1 (50)	2 (40)	5 (50)	5 (29.4)	26 (61.9)
Leukopenia					
Yes (Σ = 3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7.1)
No (Σ = 73)	2 (100)	5 (100)	10 (100)	17 (100)	39 (92.9)
Thrombocytopenia					
Yes (Σ = 1)	0 (0)	0 (0)	0 (0)	1 (5.9)	0 (0)
No (Σ = 75)	2 (100)	5 (100)	10 (100)	16 (94.1)	42 (100)
Pancytopenia					
Yes (Σ = 3)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7.1)
No (Σ = 73)	2 (100)	5 (100)	10 (100)	17 (100)	39 (92.9)
Hyponatremia					
Yes (Σ = 34)	0 (0)	0 (0)	4 (40)	8 (47.1)	22 (52.4)
No (Σ = 42)	2 (100)	5 (100)	6 (60)	9 (52.9)	20 (47.6)
Hypokalemia					
Yes (Σ = 20)	1 (50)	1 (20)	6 (60)	2 (11.8)	10 (23.8)
No (Σ = 56)	1 (50)	4 (80)	4 (40)	15 (88.2)	32 (76.2)
Hypernatremia					
Yes (Σ = 5)	1 (50)	0 (0)	0 (0)	0 (0)	2 (4.8)
No (Σ = 71)	1 (50)	5 (100)	10 (100)	17 (100)	40 (95.2)
Hyperkalemia					
Yes (Σ = 4)	0 (0)	0 (0)	0 (0)	1 (5.9)	3 (7.1)
No (Σ = 72)	2 (100)	5 (100)	10 (100)	16 (94.1)	39 (92.9)

Characteristics of bacterial and fungal identification results of HIV/AIDS-infected patients treated in the inpatient ward at Dr. Soetomo Regional Public Hospital

The gram-negative bacteria with the highest prevalence in a study by Tan *et al.* in the same hospital in the previous period were *Klebsiella pneumoniae* (35.15%), followed by *Escherichia coli* (10.8%), *Pseudomonas aeruginosa* (8.1%), and *Acinetobacter baumannii* (8.1%) [33]. Meanwhile, in this study, as depicted in Table 5, the gram-negative bacteria with the highest prevalence were *Escherichia coli* ESBL and *Pseudomonas aeruginosa*, followed by *Klebsiella pneumoniae* and *Escherichia coli*. The

gram-positive bacteria with the highest prevalence in the study by Tan *et al.* were *Streptococcus mitis/oralis* (30.7%), followed by *Staphylococcus aureus* (23.1%) and *Staphylococcus epidermidis* (15.4%) [33].

Table 5 indicates that gram-negative bacteria have a higher percentage than gram-positive bacteria. It is in line with a study conducted on inpatients with HIV/AIDS at the same hospital from August 2019 to February 2020, which stated that the most common types of bacteria were gram-negative bacteria at 44.6%, followed by gram-positive bacteria at 15.7%, while negative culture results reached 37.3% [33]. This study obtained more negative culture results at 52.5%.

Meanwhile, in this study, as displayed in Table 5, the gram-positive bacteria with the highest prevalence were *Viridans streptococci* (24.32%), followed by coagulase-negative *Staphylococci* (16.22%), *Staphylococcus haemolyticus* (13.51%), *Staphylococcus aureus* (10.81%), *Staphylococcus haemolyticus* (13.51%), *Staphylococcus hominis* (8.11%), and methicillin-resistant *Staphylococcus aureus* (5.41%). In comparison, those with the lowest prevalence of 2.70% were vancomycin-resistant *Enterococcus faecalis*, *Mycobacterium tuberculosis*, *Staphylococcus sciuri*, *Staphylococcus carnosus*, *Pediococcus pantosaceus*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Corynebacterium tuberculosis*.

In this study, the prevalence of bacteriuria/candiduria was lower at 51.16%, most of which were found in female patients (53.8%), and most were aged 25-49 years old (59.1%). The isolates with the highest prevalence

were *E. coli* ESBL at 13.95%, followed by *Pseudomonas aeruginosa* and *E. coli* at 4.65%. The prevalence of ESBL bacteria in this study was lower than that in a study conducted at Tertiary Referral Hospital in Central Europe, which stated that 16.8% of HIV/AIDS patients with positive UTIs were infected with bacteria that produce Extended-spectrum beta-lactamases (ESBLs) [34].

This study also shows that the lower the CD4 count, the higher the prevalence of bacteriuria/candiduria, which is in line with a study conducted by Tan *et al.*, which stated that HIV-infected individuals with CD4 counts of >200 cells/mm³ are less likely to experience a UTI than HIV-infected individuals with a CD4 count of <200 cells/mm³. They also stated that there was no significant correlation between other risk factors in their study [16].

Table 4: Percentage of bacteria and fungi based on specimens in HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

Bacterial and fungal profiles by specimens	N (%/SPECIMENS)	% Total specimens
Sputum specimens	69	35.94
Bacterial cultures	39 (56.5)	20.3
There was no growth of M. TB bacteria	2 (2.9)	1
There was no growth of bacteria	2 (2.9)	1
Gram-negative bacteria	18 (26.1)	9.37
<i>Acinetobacter baumannii</i>	1 (1.4)	0.5
<i>Citrobacter koseri</i>	1 (1.4)	0.5
<i>Enterobacter cloacae</i>	2 (2.9)	1
<i>Eschericia coli</i>	1 (1.4)	0.5
<i>Eschericia coli</i> ESBL	2 (2.9)	1
<i>Klebsiella pneumonia</i>	3 (4.3)	1.6
<i>Klebsiella pneumonia</i> ESBL	1 (1.4)	0.5
<i>Carbapenem-resistant Klebsiella pneumonia</i>	1 (1.4)	0.5
<i>Moraxella spp</i>	1 (1.4)	0.5
<i>Pseudomonas aeruginosa</i>	5	2.6
Gram-positive bacteria	17 (24.6)	8.8
<i>Staphylococcus aureus</i>	2 (2.9)	1
Coagulase-negative <i>Staphylococci</i>	6 (8.7)	3.1
<i>Viridans streptococci</i>	9 (13)	4.7
Fungal cultures	8 (11.6)	4.2
<i>Candida albicans</i>	7 (10.1)	3.6
<i>Candida tropicalis</i>	1 (1.4)	0.5
TB expert gene tests	22 (31.9)	11.5
Not detected <i>M. tuberculosis</i>	16 (23.2)	8.3
<i>Rifampicin-sensitive M. tuberculosis</i>	6 (8.7)	3.1
BLOOD SPECIMENS	67	34.9

There was no growth of bacteria	57 (85.1)	29.7
Gram-positive bacteria	9 (13.4)	4.7
<i>Pediococcus pentosaceus</i>	1 (1.5)	0.5
<i>Staphylococcus aureus</i>	1 (1.5)	0.5
<i>Staphylococcus haemolyticus</i>	3 (4.5)	1.6
<i>Staphylococcus hominis</i>	3 (4.5)	1.6
<i>Corynebacterium urealyticum</i>	1 (1.5)	0.5
Gram-negative bacteria	1 (1.5)	0.5
<i>Kluyvera intermedia</i>	1 (1.5)	0.5
Urine specimens	43 (100)	22.4
Bacterial cultures	42 (97.7)	21.9
There was no growth of bacteria	21 (48.9)	10.9
Gram-negative bacteria	16 (37.2)	8.3
<i>Acinetobacter baumannii</i>	1 (2.3)	0.5
<i>Citrobacter koseri</i>	1 (2.3)	0.5
<i>Eschericia coli</i>	2 (4.7)	1
<i>Carbapenem-resistant Eschericia coli</i>	1 (2.3)	0.5
<i>Eschericia coli</i> ESBL	6 (14)	3.1
<i>Klebsiella pneumoniae</i>	1 (2.3)	0.5
<i>Klebsiella pneumoniae</i> ESBL	1 (2.3)	0.5
<i>Pseudomonas aeruginosa</i>	2 (4.7)	1
<i>Proteus vulgaris</i>	1 (2.3)	0.5
Gram-positive bacteria	5 (11.6)	2.5
<i>Enterococcus faecalis</i>	1 (2.3)	0.5
<i>Vancomycin-resistant Enterococcus faecalis</i>	1 (2.3)	0.5
<i>Enterococcus faecium</i>	1 (2.3)	0.5
<i>Staphylococcus carnosus</i>	1 (2.3)	0.5
<i>Staphylococcus haemolyticus</i>	1 (2.3)	0.5
Fungal cultures	1 (2.3)	0.5
<i>Candida albicans</i>	1 (2.3)	0.5
PUS specimens	8 (100)	4.2
Gram-negative bacteria	4 (50)	2.1
<i>Eschericia coli</i>	1 (12.5)	0.5
<i>Eschericia coli</i> ESBL	1 (12.5)	0.5
<i>Pseudomonas aeruginosa</i>	2 (25)	1
Gram-positive bacteria	4 (50)	2.1
<i>Staphylococcus aureus</i>	1 (12.5)	0.5
<i>Methicillin-resistant Staphylococcus aureus</i>	2 (25)	1
<i>Staphylococcus sciuri</i>	1 (12.5)	0.5
Cerebrospinal fluid specimens	4 (100)	2.1
There was no growth of bacteria	1 (25)	0.5
<i>Staphylococcus haemolyticus</i>	1 (25)	0.5
<i>M. tuberculosis</i> (<i>M.tb</i> culture)	1 (25)	0.5
Not detected <i>M. tuberculosis</i> (gene expert)	1 (25)	0.5
Stool specimens	1 (100)	0.52
There was no growth of bacteria	1 (100)	0.5

Table 5: Bacterial and fungal identification results in all specimens in HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

Bacterial and fungal profiles by specimens	N (%/TESTS)	% Total specimens
Bacterial culture tests	160 (100)	83.3
Non-MDRO bacteria	60 (37.5)	31.25
Gram-negative bacteria	26 (16.25)	13.54
<i>Acinetobacter baumannii</i>	2 (1.25)	1.04
<i>Citrobacter koseri</i>	2 (1.25)	1.04
<i>Enterobacter cloacae</i>	2 (1.25)	1.04
<i>Eschericia coli</i>	4 (2.5)	2.08
<i>Klebsiella pneumonia</i>	4 (2.5)	2.08
<i>Kluyvera intermedia</i>	1 (0.62)	0.52
<i>Moraxella spp</i>	1 (0.62)	0.52
<i>Pseudomonas aeruginosa</i>	9 (5.63)	4.69
<i>Proteus vulgaris</i>	1 (0.62)	0.52
Gram-positive bacteria	34 (21.25)	17.89
<i>Corynebacterium urealyticum</i>	1 (0.62)	0.52
<i>Enterococcus faecalis</i>	1 (0.62)	0.52
<i>Enterococcus faecium</i>	1 (0.62)	0.52
<i>Pediococcus pantosaceus</i>	1 (0.62)	0.52
<i>Staphylococcus aureus</i>	4 (2.5)	2.08
<i>Staphylococcus carnosus</i>	1 (0.62)	0.52
Coagulase-negative <i>Staphylococci</i>	6 (3.75)	3.12
<i>Staphylococcus haemolyticus</i>	5 (3.13)	2.60
<i>Staphylococcus hominis</i>	3 (1.88)	1.57
<i>Staphylococcus sciuri</i>	1 (0.62)	0.52
<i>Streptococcus viridans</i>	9 (5.63)	4.69
<i>Mycobacterium tuberculosis</i>	1 (0.62)	0.52
MDRO bacteria	16 (0.1)	8.3
Gram-negative bacteria	13 (8.13)	6.77
<i>Eschericia coli</i> ESBL	9 (5.63)	4.69
Carbapenem-resistant <i>Eschericia coli</i>	1 (0.62)	0.52
<i>Klebsiella pneumonia</i> ESBL	2 (1.25)	1.04
Carbapenem-resistant <i>Klebsiella pneumonia</i>	1 (0.62)	0.52
Gram-positive bacteria	3 (1.88)	1.56
Vancomycin-resistant <i>Enterococcus faecalis</i>	1 (0.6)	0.52
Methicillin-resistant <i>Staphylococcus aureus</i>	2 (1.25)	1.04
There was no growth of bacteria	84 (52.5)	43.75
<i>M. tuberculosis</i> Gene Expert Tests	23 (100)	12
Not detected <i>M. tuberculosis</i>	17 (73.9)	8.88
Rifampicin-sensitive <i>M. tuberculosis</i>	6 (26.1)	3.12
FUNGAL culture tests	9 (100)	4.70
<i>Candida albicans</i>	8 (88.9)	4.18
<i>Candida tropicalis</i>	1 (11.1)	0.52

Analysis of the correlation between the CD4 counts and the bacterial and fungal profiles of HIV/AIDS-infected patients Treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

A study on urine samples of HIV/AIDS patients in Nigeria showed that CD4 counts might have little or no effect on bacteriuria. Still, with CD4 counts of ≤ 200 cells/mm³, the study subjects were more likely to show UTI symptoms even at low bacterial counts [35]. In accordance with this

study, as listed in Table 6, the UTI incidence is more common in patients with CD4 counts of <200, especially in patients with CD4 cell counts of <50.

CD4 is the target cell for HIV infection. Until now, CD4 cell counts remain an essential parameter in HIV/AIDS patients. Decreased CD4 counts in HIV infection make the patient susceptible to infection. HIV causes a cytopathic effect on CD4 by activating the immune system, resulting in PMN cell apoptosis and damage to stem cells and

lymphoid tissue, including the thymus, so that no new cells are formed. It interferes with the function and number of neutrophils. Neutrophil dysfunction makes HIV patients susceptible to bacterial infections [36]. The correlation between CD4 counts and bacterial and fungal profiles, as demonstrated in Table 6, indicated insignificant results in bivariate analysis using Spearman's rank correlation coefficient, with a p-value of 0.350.

Table 6: Bacterial and fungal profiles of HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital based on CD4 count categories

Species name	CD4 counts of > 500	CD4 counts of 200-499	CD4 counts of 100-199	CD4 counts of 50-99	CD4 counts of < 50	Total
<i>Acinetobacter baumannii</i>	0 (0)	0 (0)	0 (0)	1(2.6)	1 (0.9)	2 (1)
<i>Citrobacter koseri</i>	0 (0)	0 (0)	1 (3)	1(2.6)	0 (0)	2 (1)
<i>Enterobacter cloacae</i>	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.9)	2 (1)
<i>Eschericia coli</i>	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.7)	4 (2.1)
<i>Klebsiella pneumonia</i>	0 (0)	0 (0)	3 (9.1)	0 (0)	1 (0.9)	4 (2.1)
<i>Kluyvera intermedia</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
<i>Moraxella spp</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
<i>Pseudomonas aeruginosa</i>	1 (20)	0 (0)	0 (0)	2 (5.1)	6 (5.6)	9 (4.7)
<i>Proteus vulgaris</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
<i>Eschericia coli</i> ESBL	0 (0)	0 (0)	0 (0)	1(2.6)	8 (7.5)	9 (4.7)
Carbapenem-resistant <i>Eschericia coli</i>	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)
<i>Klebsiella pneumonia</i> ESBL	0 (0)	0 (0)	0 (0)	1(2.6)	1 (0.9)	2 (1)
Carbapenem-resistant <i>Klebsiella pneumonia</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
<i>Corynebacterium urealyticum</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
<i>Enterococcus faecalis</i>	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)	1 (0.5)
<i>Enterococcus faecium</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (0.5)
<i>Pediococcus pantosaceus</i>	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)	1 (0.5)
<i>Staphylococcus aureus</i>	0 (0)	0 (0)	0 (0)	1(2.6)	3 (2.8)	
<i>Staphylococcus carnosus</i>	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)	1 (0.5)
Coagulase-negative <i>Staphylococci</i>	0 (0)	0 (0)	1 (3)	2 (5.1)	3 (2.8)	6 (3.1)
<i>Staphylococcus haemolyticus</i>	0 (0)	1 (12.5)	0 (0)	2 (5.1)	2 (1.9)	5 (2.6)
<i>Staphylococcus hominis</i>	0 (0)	1 (12.5)	0 (0)	1(2.6)	1 (0.9)	3 (1.6)
<i>Staphylococcus sciuri</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
<i>Streptococcus viridans</i>	0 (0)	0 (0)	2 (6.1)	1(2.6)	6 (5.6)	9 (4.7)
<i>Mycobacterium tuberculosis</i>	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.9)	1 (0.5)
Vancomycin-resistant <i>Enterococcus faecalis</i>	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)	1 (0.5)

Methicillin-resistant <i>Staphylococcus aureus</i>	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.9)	2 (1)
Sterile	3 (60)	4 (50)	16 (48.5)	19 (48.7)	42 (39.3)	84 (43.8)
Rifampicin-sensitive <i>M. tuberculosis</i>	0 (0)	0 (0)	1 (3)	3 (7.7)	2 (1.9)	6 (3.1)
Not detected <i>M. tuberculosis</i>	0 (0)	2 (25)	3 (9.1)	1(2.6)	11 (10.3)	17 (8.9)
<i>Candida albicans</i>	0 (0)	0 (0)	1 (3)	3 (7.7)	4 (3.7)	8 (4.2)
<i>Candida tropicalis</i>	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)	1 (0.5)
Total	5 (100)	8 (100)	33 (100)	39 (100)	107 (100)	192 (100)

The bivariate analysis showed an insignificant correlation between the CD4 cell counts and the bacterial and fungal profiles, with a p-value of 0.350.

Analysis of the correlation between the CD4 counts and the positive values of bacterial and fungal identification of HIV/AIDS-infected patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

The correlation between CD4 counts and the positive values of bacterial or fungal identification, as provided in Table 7, also revealed insignificant results in bivariate analysis using Spearman's rank correlation coefficient, with a p-value of 0.238.

Some studies show a lack of association between CD4 and bacteriuria. For instance, a study in Nigeria noticed that bacteriuria in HIV-infected women was not affected by CD4 counts [37], bacteriuria was not affected by CD4 counts of less than 200 cells/mm³ [38], and CD4 cell counts of <200 cells/mm³ does not affect the occurrence of asymptomatic bacteriuria [39]. Other studies yielded different results in that bacteriuria was found to correlate to CD4 cell counts and/or viral loads [40-42].

Bacteriuria may not be affected by CD4 counts because research shows that immunity to UTIs results from complex interactions between host factors and the invading organism. Defenses against UTIs include urinary pH, urinary flushing

effect, high salt concentration, chemical inhibition secreted by the epithelial cells lining the urinary tract, secretory IgA, and lactoferrin activity (an iron-binding protein that absorbs iron, invasion of starved bacteria that causes decreased growth rate). The prostate gland in men secretes an infection-fighting substance. The epithelial cells lining the urinary tract also secrete antimicrobial peptides that directly kill bacteria. The mucous lining of the bladder contains antimicrobial substances that help eliminate invading pathogens. This defense mechanism is not influenced by the host's CD4 counts. This partly explains why individuals with CD4 cell counts of less than 100 cells/mm³ can have sterile cultures, despite the lack of exposure to UTI causative agents can be responsible for that. It suggests that the host's CD4 counts may have little or no effect on bacteriuria [35].

The neutrophil-to-lymphocyte ratio (NLR) is a simple parameter utilized to assess the inflammatory status and results from the division of total neutrophils by total lymphocytes [43]. NLR is a marker of inflammation and HIV severity [19]. Table 1 presents the highest average NLR values in the 50-99 CD4 count range and the lowest in the 100-199 count CD4 range. The average NLR value varies in different CD4 count ranges, which can be caused by the administration of antibiotics received by the patients.

Table 7: Positive values of bacterial and fungal identification based on the CD4 count categories

Bacterial and fungal profiles	CD4 counts of > 500 n (%)	CD4 counts of 200-499 n (%)	CD4 counts of 100-199 n (%)	CD4 counts of 50-99 n (%)	CD4 counts of < 50 n (%)	Total n (%)
Results (+)	3 (60)	6 (75)	19 (57.6)	21 (53.8)	53 (49.5)	102 (53.1)
Results (-)	2 (40)	2 (25)	14 (42.4)	18 (46.2)	86 (48)	90 (46.9)
Total	5 (100)	8 (100)	33 (100)	39 (100)	107 (100)	192 (100)

The bivariate analysis revealed an insignificant correlation between the CD4 cell counts and the positive bacterial or fungal identification values, with a p-value of 0.238.

Analysis of the correlation between the NLR values and the bacterial and fungal profiles of HIV/AIDS-infected patients treated in the special inpatient

ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

The correlation between NLR value and bacterial and fungal profiles, as shown in Table 8, indicated insignificant results in bivariate analysis using Spearman's rank correlation coefficient, with a p-value of 0.956.

Table 8: Bacterial and fungal profiles based on NLR values

Bacterial and fungal profiles	NLR
There was no growth of bacteria	11.5 + 9.80
Gram-positive bacteria	10.14 + 7.67
Gram-negative bacteria	13.09 + 13.56
MDRO	9.67 + 5.11
Rifampicin-sensitive <i>M. tuberculosis</i>	10.07 + 5.15
Not detected <i>M. tuberculosis</i>	8.23 + 5.86
Fungus	7.13 + 4.63

The bivariate analysis showed an insignificant correlation between the NLR values and the bacterial and fungal profiles, with a p-value of 0.557.

Analysis of the correlation between the NLR values and the positive values of bacterial and fungal identification of HIV/AIDS patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital

The correlation between NLR value and the positive values of bacterial or fungal identification, as illustrated in Table 9, also revealed insignificant results in bivariate analysis using Spearman's rank correlation coefficient, with a p-value of 0.557. This study shows that the bacterial and fungal profiles have no significance to the NLR values because, as depicted in Table 1, 82.8% of the isolates were exposed to antibiotics. Only 17.2% of isolates were not exposed to

antibiotics, even though antibiotics could not quickly turn the results of the gene expert TB tests into negative. In addition, the gene expert tests in this study only account for 12%, so it can be concluded that the antibiotics administration can affect the NLR values. Commensal bacteria at 34.2% in the bacterial culture results indicated that the bacteria obtained were normal flora, even though they can serve as causative agents in immunocompromised patients.

NLR is affected by many conditions, including age, race, medications, and chronic diseases, such as coronary heart disease, stroke, diabetes, obesity, psychiatric diagnosis, solid organ cancer, anemia, and stress [43]. In Asian races, NLR values will usually be higher in women than those in men because estrogen can inhibit apoptosis. Older people, especially women, have lower NLR values due to decreased estrogen due to menopause [44, 27].

Table 9: Positive values of bacterial and fungal identification based on the average NLR value

Bacterial and fungal profiles	NLR
Bacterial and fungal identification (+)	9.71 ± 7.19
Bacterial and fungal identification (-)	10.80 ± 9.85

The bivariate analysis indicated an insignificant correlation between the NLR values and the positive values of bacterial and fungal identification of HIV/AIDS-infected patients treated in the inpatient ward at Dr. Soetomo Regional Public Hospital, with a p-value of 0.956.

The distribution of HIV/AIDS-infected patients in the samples of this study showed that male patients had a dominant percentage of 77.6%. It differs from the data in global HIV statistics issued by UNAIDS, which stated that 54% of HIV/AIDS-infected people were women and girls [23]. Still, the percentage of male patients in this study was slightly higher than that in the 2020 HIV data from the Data and Information Center of the Ministry of Health of the Republic of Indonesia, which stated that the percentage of male HIV-infected patients reached 64.5% and that of male AIDS-infected patients reached 68.60% [24]. The percentage of male patients is higher than that of female patients because men are more likely to have risky sexual intercourse than women and more likely to use illegal drugs (injecting needles) [25].

Most HIV/AIDS patients in this study were aged 25-49 years old, amounting to 72.4%. It is higher than that in the data and information center of the Ministry of Health of the Republic of Indonesia in 2021, which states that most HIV patients come from the age range of 25-49 years old, amounting to 69.7% [24]. UNAIDS also states that men aged 25-49 occupy the largest population at 42% [26]. The percentage of HIV/AIDS patients aged 25-49 years old in this study was higher than that in data from the Ministry of Health and UNAIDS because this study only included adult inpatients. In comparison, data from the Ministry of Health and UNAIDS included all patients from 0 year old. The age range of 25-49 years old is a productive age, and it is very likely for those in this age range to get infected with HIV due to high sexual activity.

Conclusion

According to the profiles of HIV/AIDS patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Tertiary Referral Hospital from September 2021 to August 2022, most of them were male patients at 77.6% aged 20-24 years old at 13.2%, with the highest HIV stage being the AIDS stage at 90.8%. The highest OIs included pneumonia at 28.9%, followed by pulmonary TB at 23.7% and oral candidiasis at 21.1%. The bacterial culture tests of 83.3% showed 52.5% negative cultures, 47.5% positive cultures, and 51.33% gram-negative bacteria, with *Pseudomonas aeruginosa* and *Escherichia coli* ESBL as the highest number of isolates, 48.67% gram-positive bacteria with *Streptococcus viridans* as the highest number of isolates, 34.2% commensal bacteria, 65.8% pathogenic bacteria, and 21.05% MDRO bacteria. The TB expert gene tests of 12% resulted in 73.9% undetected *M. tuberculosis* and 26.1% positive, rifampicin-sensitive *M. tuberculosis*, while the fungal culture tests of 4.7% resulted in 88.9% *Candida albicans* and 11.1% *Candida tropicalis*. There is no correlation between the profile of bacteria and fungi from the clinical specimens with CD4 counts and NLR values of HIV/AIDS patients treated in the special inpatient ward for HIV/AIDS isolation at Dr. Soetomo Regional Public Hospital from September 2021 to August 2022. The profiles of HIV/AIDS patients in this study did not describe all the patients as a whole because the data were collected only from patients with complete medical records of CD4 counts and NLR values.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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