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Relationship between respiratory tract microbiota dysbiosis and serum procalcitonin levels and clinical outcomes in patients with pneumonia

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Abstract

Lower respiratory tract infections remain a major problem worldwide. One biomarker that has long been used and is still used today is procalcitonin. Microbiota dysbiosis is a condition in which there is a shift in the number and proportion of the normal flora balance in the respiratory tract. However, to date, there have been few studies discussing respiratory tract dysbiosis in relation to serum procalcitonin levels and clinical outcomes in pneumonia patients. Lower respiratory tract infections remain a major problem worldwide. To analyze the relationship between respiratory tract microbiota dysbiosis and serum procalcitonin levels and clinical outcomes in pneumonia patients. This study is an observational analytical study with a cross-sectional design involving 19 patients with pneumonia on mechanical ventilation in the intensive care unit of Dr. Soetomo General Hospital in Surabaya who met the study criteria. All samples were obtained between October 2024 and August 2025. An assessment of respiratory tract microbiota dysbiosis was performed on serum procalcitonin levels and clinical outcomes using BAL fluid samples No significant relationship was found between respiratory tract microbiota dysbiosis, as assessed using alpha diversity index, and serum procalcitonin levels and clinical outcomes, as determined using Spearman's rho statistical test, Mann-Whitney, and independent T-tests, with a statistical test value of $p<0.05$ and displayed in a Bloxpot statistical curve. The assessment of respiratory tract microbiota dysbiosis could not show a correlation with serum procalcitonin levels and clinical outcomes.

Keywords: Pneumonia, Microbiota dysbiosis, Procalcitonin

Introduction

Lower respiratory tract infections remain a major problem worldwide. According to *the Global Burden* of *Disease* in 2019, the incidence of lower respiratory tract infections, including pneumonia, reached 489 million cases with a mortality rate of 2.5 million cases annually. Pneumonia also ranks 10th among the leading causes of death in Indonesia(Abbafati et al., 2020) . The incidence of pneumonia is generally more common in developing countries than in developed countries. Every year, there are 450 million reported cases of pneumonia. Based on RISKESDAS in 2018, the prevalence of pneumonia based on diagnosis by health workers was around 2%, while in 2013 it was 1.8%(Kemenkes, 2018) .

In diagnosing patients with pneumonia, biomarkers with good specificity are needed. One biomarker that has been used for a long time and is still used today is procalcitonin. Currently, procalcitonin has been developed into a biomarker for the early detection of systemic bacterial infections, including pneumonia. Procalcitonin itself is a polypeptide consisting of 116 amino acids with a molecular weight of 14.5 kDa. The procalcitonin component consists of three parts, namely the amino terminal consisting of 57 amino acids, immature calcitonin consisting of 33 amino acids, and calcitonin carboxyl-terminal peptide 1 (CCP-1), also known as catacalcin, consisting of 21 amino acids(Cleland et al., 2023) .

The mechanism of pneumonia and changes to the microbiota ecosystem require further study. To date, studies on the microbiota conducted on the lungs can reveal new insights and provide detailed explanations and understanding of the pathogenesis of lung disease. The normal flora of the respiratory tract plays many roles, such as nutrient absorption, vitamin synthesis, xenobiotic metabolism, and immune regulation, which have been linked to various problems such as infection, autoimmunity, obesity, cardiovascular disease, and malignancy. Analysis of the microbiota can identify bacteria down to the genus and species level using *Next-Generation Sequencing* methods through the identification of bacteria that detect variations in the 16S rRNA gene, a small and conserved locus of the bacterial genome. In a study conducted by Pascale et al. in Italy in 2019 on the incidence of COVID-19 pneumonia, an increase

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in procalcitonin was found to be associated with an increase in the number of Proteobacteria, and a decrease in procalcitonin was associated with an increase in Firmicutes(Dickson et al., 2013; Cho & Blaser, 2012; De Pascale et al., 2024) .

Microbiota dysbiosis is described as a condition in which there is a shift in the number and proportion of the normal flora balance of a mutualistic symbiotic life that occurs in the respiratory tract (Yang et al., 2020) . One key to effectively evaluating microbiota dysbiosis is the use of appropriate sampling techniques. Commonly performed actions and procedures include both invasive and non-invasive sampling methods. Invasive methods can provide more accurate results and help identify the source of pathogens causing infections in the lower respiratory tract, but these procedures require the expertise of a pulmonologist. Non-invasive methods, on the other hand, are commonly used when a patient is suspected of having a respiratory tract infection(Asty et al., 2019) .

The most common invasive procedure used to diagnose lower respiratory tract infections is *bronchoalveolar lavage* (BAL). BAL is defined as a minimally invasive procedure involving the instillation of sterile normal saline into the lungs, followed by suctioning, and then collecting the instilled fluid for analysis(Pate et al., 2022) . Due to the limited use of microbiota examination as a diagnostic tool to evaluate lower respiratory tract infections in pneumonia patients on mechanical ventilation, this study aims to demonstrate the relationship between dysbiosis in microbiota and serum procalcitonin levels, as well as clinical outcomes (whether patients survive or die during *intensive care* unit treatment*).*

Research Method

This study is an observational analysis to examine the relationship between microbiota dysbiosis in pneumonia patients, serum procalcitonin levels, and clinical outcomes in the ICU, ROI, and RES at Dr. Soetomo General Hospital in Surabaya. The study design used was *cross-sectional* and prospective cohort. The study population consisted of all pneumonia patients on mechanical ventilation treated in the ICU, ROI, and RES at Dr. Soetomo General Hospital in Surabaya from October 2024 to August 2025. The study sample consisted of of pneumonia patients on mechanical ventilation treated in the ICU, ROI, and RES at Dr. Soetomo General Hospital in Surabaya from October 2024 to August 2025 who met the inclusion and exclusion criteria.

The sample was taken from pneumonia patients using mechanical ventilators who were eligible for bronchoscopy. The estimated minimum sample size formula is 19 people. Therefore, the required sample size is 19-21 people. Research subjects were taken using *consecutive sampling* of patients who met the inclusion and exclusion criteria and were included in the study until the sample size was met. The inclusion criteria for this research sample were:

1. Patients with pneumonia who used mechanical ventilators in the ICU, ROI, and RES wards of Dr. Soetomo General Hospital from October 2024 to August 2025.
2. Aged over 18 years at the time of bronchoscopy.
3. Research subjects (family/guardians of subjects) who are willing to sign the *informed consent* form to participate in the study.

Materials for bronchoalveolar lavage examination, data collection forms, fiber optic bronchoscopy (FOB), transport media, containers for storing BAL specimens. Equipment and materials for Next-Generation 16S Amplicon sequencing, freezer (-200°C, -800°C), permanent markers, sterile gloves, absolute ethanol, centrifuge for 96-well PCR plates 0.2 mL (≤ 4000 x g), microcentrifuge (20,000 x g), PCR thermal cycler, 50 mL graduated conical-bottom sterile tubes with rack, sterile 1.5 mL microcentrifuge tubes, adjustable pipettes (1000-µl, 200-µl, and 10-µl), disposable aerosol filter pipette tips (1000-µl, 200-µl, and 10-µl), spray bottles with 70% ethanol, spray bottles with 10% bleach, Illumina Miseq Set, MiSeq Reagent kit V3.

The research was conducted in the *Intensive Care Unit* of the Integrated Surgical Center (ICU GBPT), Resuscitation Room (RES), and Intensive Observation Room (ROI) of Dr. Soetomo General Hospital. Microbiota examination using the 16S rRNA PCR method to determine the alpha diversity index was

conducted at the *Institute of Tropical Disease* (ITD) Laboratory, Airlangga University, in collaboration with the GSI Laboratory in Jakarta. The study period was from October 2024 to August 2025.

Subjects (families/guardians of patients) who will be sampled for the study must receive complete information about the examination to be performed. If the family/guardian of the patient agrees to participate in this study, they will sign an *informed* consent form. To maintain patient confidentiality, initials or a numbering system known only to the research team will be used. The research is conducted based on the principles of GCP (*Good Clinical Practice*). This research does not involve any *conflict of interest* from the sponsoring organization or company. This research began after approval from the ethics committee of Dr. Soetomo General Hospital in Surabaya.

Data obtained during the study will be analyzed and presented in the form of distribution and frequency tables. Primary data will be edited, *coded*, and *entered* into a computer using the SPSS 26.0 *for* MacOS statistical program (SPSS Inc, Chicago). The research will be analyzed using *Chi Square*, and the strength of the relationship will be analyzed using the contingency coefficient. The interpretation of the results is based on *the p-value*; statistical analysis is considered significant if $p<0.05$.

Results: General characteristics of subjects in the study: The general characteristics of the subjects in this study included gender, age, body mass index, comorbidities, other risk factors for pneumonia, and type of pneumonia diagnosis. Complete data can be seen in Table 1.

The general characteristics of the study subjects showed that the majority were male (73.6%) with a mean age of 45 years and a range of 18–81 years. The mean body mass index (BMI) of the subjects was 25.53 ± 4.93 kg/m^2, with most falling into the normal BMI category () (52.6%). A total of 11 subjects (57.8%) had comorbidities, including hypertension (10 subjects), diabetes mellitus (3 subjects), neuromuscular disorders such as myasthenia gravis, Guillain-Barre syndrome, and Becker Muscular Dystrophy (3 subjects), and stroke (1 subject). The diagnosis of pulmonary infection varied, with 9 subjects (47.3%) having HAP, 8 subjects (42.1%) having CAP, and 2 subjects (10.6%) having VAP. All subjects who underwent BAL sampling had received empirical antibiotics (100%), while 3 subjects (15.8%) had received steroid therapy and 16 subjects (84.2%) had not received steroid therapy.

Table 1. General characteristics of research subjects

Characteristics	Results, n (%)
Gender Male Female	 14 (73.6) 5 (26.4)
Age (years), mean ± SD 18–20	44.63 20.45
21 - 30	2 (10.6)
31 - 40	4 (21.0)
41 - 50	3 (15.8)
51	4 (21.0)
61	1 (5.3)
71 - 80	1 (5.3)
>80	3 (15.8)
	1 (5.3)
Body Mass Index (kg/m^2), mean SD	25.53 ± 4.93
<18.50	0 (0)
18.50 - 24.99	10 (52.6)
25.0 - 29.99	7 (36.8)
30.0 - 34.9	1 (5.3)
>34.9	1 (5.3)
History of Comorbidities*	
None	8
Yes:	11
Hypertension	7
Diabetes	3
Chronic kidney failure	1
Stroke	1
Neuromuscular disorders	3
Other	2
Diagnosis of CAP Pneumonia HAP VAP	 8 (42.1) 9 (47.3) 2 (10.6)
Administration of antibiotics during BAL collection Empirical antibiotics have been administered Empirical antibiotics not yet administered	 19 0
Administration of steroids Steroids administered No steroids administered	 3 (15.8) 16 (84.2)

* More than one comorbidity may be found in a single research subject

Respiratory tract microbiota dysbiosis in pneumonia patients

The condition of microbiota dysbiosis in 19 research subjects was assessed through several alpha diversity indices, namely the Richness Index, Simpson's Index, Shannon Diversity Index, Pielou Evenness Index, and Chao1 Index. The Richness Index, which describes the number of species/OTUs in a sample without considering their proportions, showed a minimum value of 2, a maximum value of 223, a median of 59, and an average of 73 ± 61.704, indicating an increase in the richness of microbiota species in the respiratory tract. The Simpson's Index, which describes the probability that two random individuals belong to the same species, had a median of 0.63 and an average of 0.61 ± 0.21, indicating that the majority of subjects had low species ecosystem diversity.

Meanwhile, the Shannon Diversity Index reflects the level of uncertainty when taking one individual at random from a community, with a median of 1.85 and an average of 1.83 ± 0.83, indicating that the distribution between species is still low compared to the ideal value of 3.5. The Pielou Evenness Index, which measures how evenly individuals are distributed among species, showed a median of 0.54 and an average of 0.50 ± 0.202, with approximately 42.4% of subjects showing dominance of certain microbiota.

The Chao1 Index, which assesses total taxon richness including rare species, had identical values to the Richness Index (median 59, mean 73 ± 61.704), indicating no rare species or unseen diversity in the subjects' microbiota communities. These results indicate that among the 19 study subjects, there was variation in the richness and distribution of respiratory tract microbiota, with a tendency for some subjects to have low species diversity and balance.

Serum procalcitonin levels in pneumonia patients

Serum procalcitonin levels were measured in 19 study subjects who underwent BAL and met the inclusion criteria, and were divided into 4 assessment levels as listed in Table 2.

Table 2. Serum procalcitonin levels

Serum procalcitonin levels (ng/mL)	Results, n (%)
<0.1	3 (15.8)
0.1 – 0.25	4 (21.1%)
>0.25	1 (5.3%)
>0.5	11 (57.9%)
Total	19 (100%)

In this study of 19 subjects, the most common distribution of serum procalcitonin levels was found in subjects with serum procalcitonin levels > 0.5 (ng/mL), accounting for 11 subjects (57.9%), as shown in Table 2. The results of serum procalcitonin testing revealed a median value of 1.25, a minimum value of 0.02, and a maximum value of 29.40.

The relationship between respiratory tract microbiota dysbiosis and serum procalcitonin levels in pneumonia patients

All data on the dysbiosis of the microbiota of the research subjects were tested for normality using *the Shapiro-Wilk* test for serum procalcitonin levels, and the serum procalcitonin data distribution value was found to be $p < 0.05$, so it was concluded that the data values of these two indices had a non-normal distribution. *Spearman's rho* test was used to analyze the correlation between microbiota dysbiosis and serum procalcitonin levels. In this test, none of the alpha diversity indices had a significant relationship with serum procalcitonin levels. The results *of Spearman's rho test* are shown in Table 3.

Table 3. *Spearman's rho* test to assess the relationship between respiratory tract microbiota dysbiosis and serum procalcitonin levels in pneumonia patients.

***Spearman's* Test**	**Correlation coefficient**	**p**
Richness index	-0.252	0.298
Simpson's index	-0.652	0.800
Shannon diversity index	-0.159	0.516
Pielou evenness index	0.215	0.377
Chao1	-0.252	0.298

The results of Spearman's rho test from Table 3 show that all alpha diversity indices have no significant relationship with serum procalcitonin levels. The correlation coefficients ranged from -0.652 for Simpson's Index to 0.215 for Pielou Evenness Index,

with p-values ranging from 0.298 to 0.800, indicating no evidence of a correlation between the level of respiratory tract microbiota dysbiosis and serum procalcitonin levels in pneumonia patients.

Relationship between respiratory tract microbiota dysbiosis and clinical outcomes in pneumonia patients: All data on the microbiota dysbiosis of the research subjects underwent a *Shapiro-Wilk* normality test for clinical outcome categories, and the data distribution values for microbiota dysbiosis on three indices, namely *the Simpson's index, Shannon diversity index, and Pielou evenness index*, were p > 0.05, so it was concluded that the data values for these three indices had a normal distribution.To analyze the correlation between microbiota dysbiosis using these three indices and clinical outcomes, an *independent T-test* was used.

Table 4. Independent T-test for microbiota dysbiosis and clinical outcome on the richness index

T-test	n	Mean± SD	p
Wealth index			
Life	9	73,± ,67,285	1,000
Death	10	73,± ,59,922	

Table 5. Independent T-test for microbiota dysbiosis and clinical outcomes on the Simpson index

T-test	n	Mean± SD	p-value
Simpson's index			
Alive	9	0.605± 0.220	0.895
Death	10	0.619± 0.231	

Table 6. Independent T-test for microbiota dysbiosis and clinical outcomes on the Shannon diversity index

T-test	n	Mean± SD	p-value
Shannon diversity index	9	1,822± 0.856	0.934
Alive	10	1,854± 0.851	
Dead			

Table 7. Independent T-test for microbiota dysbiosis and clinical outcomes on the Pielou evenness index

T-test	n	Mean± SD	p-value
Pielou evenness index	9	0.500± 0.221	0.943
Alive	10	0.507± 0.195	
Death			

Table 8. Independent T-test for microbiota dysbiosis and clinical outcomes on the Chao1 index

T-test	n	Mean± SD	p-value
Chao1 index			
Alive	9	73,± ,67,285	1,000
Death	10	73,± ,59,922	

Based on the results of the independent T-test, no significant relationship was found between microbiota dysbiosis and clinical outcomes in all alpha diversity indices, including *the Richness Index, Simpson's Index, Shannon Diversity Index, Pielou Evenness Index, and Chao1 Index*, with p-values ranging from 0.895 to 1.000. Shapiro-Wilk analysis showed that the distribution of microbiota dysbiosis data on *the Richness Index* and *Chao1 Index* was not normal (p < 0.05), so the Mann-Whitney test was performed for these two indices.

The Mann-Whitney test results still showed no significant relationship with clinical outcomes (p = 0.967), so it can be concluded that the level of microbiota dysbiosis, based on *the Richness Index, Chao1 Index,* and other alpha diversity indices, has no relationship with clinical outcomes in the patients in this study.

Discussion

This study analyzed the relationship between respiratory tract microbiota dysbiosis and serum procalcitonin levels as well as clinical outcomes in 19 pneumonia patients treated in the ICU at Dr. Soetomo General Hospital in Surabaya. The study findings showed diverse subject characteristics, with ages ranging from 18 to 81 years, and the largest age groups were 21–30 years and 41–50 years, each with 8 subjects (42%). Although pneumonia is epidemiologically more common in the elderly population, the relatively high incidence in the younger age group in this study may be influenced by predisposing factors such as neuromuscular disorders, malignancies, or severe trauma, which increase susceptibility to pneumonia(Shi et al. 2020)

In terms of gender, the majority of study subjects were male (73.6%), consistent with previous epidemiological studies showing a higher risk of pneumonia in males compared to females, likely due to behavioral factors such as smoking history(

Baskaran et al., 2019; Rivero-Calle et al., 2016) . These findings are in line with reports from Spain (and Spain (de Miguel-Yanes (2021))which showed that 54–59% of pneumonia patients ( ) were male. This condition confirms that gender and behavioral factors play an interactive role in determining the risk of pneumonia.

The Body Mass Index (BMI) of the study subjects was predominantly in the normal category (52.6%), indicating that BMI did not significantly influence the incidence of pneumonia in this study. These findings are consistent with those of Borisov et al. (2022) , which show that BMI does not always correlate with the risk or clinical outcomes of pneumonia, although a meta-analysis by Phung et al. (2013) and a population-based study in Denmark indicate variations in risk based on BMI categories(Kornum et al., 2010) .

Comorbid factors also play an important role in this study. A total of 57.8% of subjects had comorbidities, including hypertension, diabetes mellitus, chronic kidney disease, stroke, and neuromuscular disorders. The risk of pneumonia increases significantly in patients with certain comorbidities, such as chronic lung disease, diabetes, or immune disorders(. Campling et al., 2019). The pathophysiological mechanisms involve endothelial dysfunction, chronic inflammation, immune dysregulation, and respiratory dysfunction in patients with neuromuscular diseases such as *Myasthenia Gravis, Guillain–Barré Syndrome*, and *Becker Muscular Dystrophy*(. Su et al., 2022; Maskin et al., 2024) .

Microbiota dysbiosis analysis showed variations in microbiota richness and distribution in 19 study subjects. Based on the alpha diversity index, *Richness Index* and *Chao1 Index* showed an increase in microbiota species richness in some subjects (52.6%), while *Simpson's Index* and *Shannon Diversity Index* showed a tendency toward low species distribution. *The Pielou Evenness Index* showed microbiota dominance of 42.2%, indicating uneven distribution. These findings indicate that despite variations, most subjects exhibited microbiota dysbiosis reflecting microbial community imbalance(Avalos-Fernandez et al., 2022; Finn, 2024) .

The relationship between microbiota dysbiosis and serum procalcitonin levels did not show significant results. Factors influencing these findings include the use of empirical antibiotics in all subjects, which is known to alter the composition and reduce the diversity of the microbiota, as well as a history of steroid use, which can lower procalcitonin levels through its immunomodulatory effects(Samsudin & Vasikaran, 2017; Kitsios et al., 2018; Martin-Loeches et al., 2020) . Additionally, prolonged mechanical ventilation increases the risk of microaspiration and microbiota translocation, meaning that BAL samples may not reflect the initial microbiota condition at the time of pneumonia diagnosis.

Furthermore, no significant association was found between microbiota dysbiosis and clinical outcomes, including mortality and length of stay. Contributing factors include subject population heterogeneity, comorbidity variation, and the influence of antibiotics and steroids, which can modify microbiota composition, such that alpha diversity scores do not reflect actual microbiota conditions(Dickson et al., 2020; Carney et al., 2020) .

The findings of this study confirm that in critically ill pneumonia patients undergoing mechanical ventilation and complex medical interventions, the effect of microbiota dysbiosis on serum procalcitonin levels and clinical outcomes is difficult to determine significantly. Individual variability, the use of empirical antibiotics, steroid therapy, and clinical heterogeneity are important factors that must be considered in interpreting respiratory tract microbiota research results(Alagna et al., 2023) . Thus, these findings provide insight into the limitations of microbiota dysbiosis in critical care and do not confirm a direct relationship with clinical outcomes.

Conclusion

This study shows that in pneumonia patients admitted to the intensive care unit, respiratory tract microbiota dysbiosis can occur with variations observed in various alpha-diversity indices, including *Richness, Simpson, Shannon, Pielou,* and *Chao1,* although these changes are not significantly associated with serum procalcitonin levels or clinical outcomes. These findings indicate that although microbiota imbalance is present in critically ill

patients, other factors such as comorbidities, empirical antibiotic use, steroid therapy, and the duration and timing of mechanical ventilation may influence the relationship between microbiota and clinical indicators, so interpretation of the results must consider the complexity of the patient's clinical context.

The limitations of the study include delayed sampling due to the clinical condition of the patients and the absence of specific BAL SOPs, as well as limited molecular facilities for *Next-Generation Sequencing* and PCR16sRNA testing in Surabaya. For future research, it is recommended to evaluate microbiota dysbiosis before administering antibiotics, use other infection parameters and more diverse clinical outcomes, and strengthen interdepartmental collaboration to improve the quality of microbiota analysis. This approach is expected to provide a more accurate understanding of the role of microbiota in the prognosis of critically ill pneumonia patients and support more targeted management strategies.

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