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The relationship between biofilm production capacity with clinical outcomes and antibiotic resistance of Gram-Negative bacteria of pneumonia patients on mechanical ventilation

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Abstract

Pneumonia is a public health issue with a broad impact on quality of life, productivity, and social and economic aspects, ranking among the leading causes of hospitalization in Indonesia. Gram-negative bacteria that because pneumonia are capable of producing biofilms. Biofilms play a crucial role in pathogenesis and antimicrobial resistance because they protect bacteria from antibiotic therapy and contribute to treatment failure, thereby potentially affecting patients' clinical outcomes. The correlation between biofilm-producing capacity, clinical outcomes, and antibiotic resistance of Gram-negative bacteria has not yet been extensively investigated, indicating the need for further research. Objective. To analyze the relationship between biofilm production capacity, clinical outcomes, and antibiotic resistance of Gram-negative bacteria of pneumonia patients on mechanical ventilation. Methods. This was an observational analytic study with a cross-sectional design involving 20 mechanically ventilated pneumonia patients in the intensive care unit of Dr. Soetomo General Hospital, Surabaya, who met the study criteria. Samples were collected between February and August 2025. The biofilm production capacity of Gram-negative bacteria was assessed in relation to clinical outcomes and antibiotic resistance using bronchoalveolar lavage (BAL) fluid samples. Results. No significant relationship was found between the biofilm production capacity of bacteria and clinical outcomes or antibiotic resistance of Gram-negative bacteria, as determined by Chi-square or Fisher's exact statistical test with a p-value >0.05. Conclusions. The assessment of biofilm production capacity does not show a correlation with clinical outcomes or antibiotic resistance in Gram-negative bacteria.

Keywords: Pneumonia, Biofilm, Clinical outcomes, Resistance, Gram-Negative.

Methods

This study is an observational analytical study with a cross-sectional design. The study was conducted in the Intensive Care Unit of Dr. Soetomo General Hospital in the period between February and August 2025. The study sample was pneumonia patients with mechanical ventilation who met the inclusion and exclusion criteria. We investigated 20 BAL samples.

Inclusion criteria: adult patients (≥18 years old) diagnosed with pneumonia and receiving mechanical ventilation with PEEP <10 cmH_2O or FiO_2 <40% in the intensive care unit of Dr. Soetomo General Hospital, BAL samples that yield Gram-negative bacterial isolates, specifically Klebsiella pneumoniae, Acinetobacter baumannii, or Pseudomonas aeruginosa, and patients or their legal guardians who provide written informed consent to participate in the study.

Exclusion criteria: patients with other pulmonary infections, including pulmonary tuberculosis or pulmonary mycosis and patients with contraindications to bronchoscopy, such as myocardial infarction or angina, cardiac arrhythmias, or impaired hemostatic function. The process of identifying Gram-negative bacteria was performed using a BD PhoenixTM automated identification and susceptibility testing system. The biofilm production capacity was performed using TCP (Tissue Culture Plate) method.

Statistical analysis

Data are presented as frequency distributions. Statistical tests were considered significant if p<0.05. The association between biofilm production capacity and the clinical outcomes and antibiotic resistance of Gram-negative bacteria was analyzed using the Chi-square test or Fisher's exact test. The influence of clinical characteristics of the study subjects on the biofilm production ability of Gram-negative bacteria

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was analyzed using the Chi-square test or Fisher's exact test and the Kruskal–Wallis H test. Factors associated with clinical outcomes were analyzed using the Chi-square test or Fisher's exact test and the Mann–Whitney test. Interpretation of results was based on p-values, with statistical significance defined as $p < 0.05$.

Ethical clearance

This study has received approval for ethical clearance by the Health Research Ethics Committee of Dr. Soetomo General Hospital Surabaya number 1221/KEPK/II/2025.

Results

Table 1. Comparison of clinical characteristics of study subjects according to the biofilm production levels of Gram-negative bacteria

Clinical charactheristics	Biofilm production capacity			p
	Weak producer (n = 4)	Moderate producer (n = 13)	Strong Producer (n = 3)	
Median of patient's age (years)	50 (23 - 81)	33 (18 - 73)	52 (22 - 79)	0,416
Sex				0,424
Male	3 (75%)	7 (53,8%)	3 (100%)	
Female	1 (25%)	6 (46,2%)	0 (0%)	
Median of BMI (kg/m^2)	26,8 (23,0 -28,3)	23,6 (19,4 -33,2)	23,4 (23,4 -27,7)	0,37
Comorbidities				0,224
No	0 (0%)	6 (46,2%)	2 (66,7%)	
Yes	4 (100%)	7 (53,8%)	1 (33,3%)	
Pneumonia				0,527
CAP	1 (25%)	5 (38,4%)	2 (66,7%)	
HAP	2 (50%)	2 (15,4%)	1 (33,3%)	
VAP	1 (25%)	6 (46,2%)	0 (0%)	
Initial admission to intensive care unit				0,346
No	3 (75%)	4 (30,8%)	1 (33,3%)	
Yes	1 (25%)	9 (69,2%)	2 (66,7%)	
Median of duration from admission to infection (days)	28,5 (5 - 43)	14,0 (7 - 30)	5 (3 - 23)	0.353
Median of APACHE II score	14 (7 - 22)	11 (3 - 27)	19 (17 - 24)	0,183
Median of time of specimen collection after administration of empirical antibiotics (days)	6 (3 - 15)	8 (2 - 15)	2 (2 - 3)	0,049

Table 2. The relationship between biofilm production capacity and clinical outcomes

Biofilm production capacity	Clinical outcomes		*p*
	Alive n (%)	Deceased n (%)	
Weak producer	2 (20)	2 (20)	0,276
Moderate producer	8 (80)	5 (50)	
Strong producer	0 (0)	3 (30)	

Statistical analysis using the Chi-square test or Fisher's exact test and the Kruskal-Wallis H test demonstrated a statistically significant difference in the variable of specimen collection time after empirical antibiotic administration ($p < 0.05$).

The association between the biofilm production ability of Gram-negative bacteria and the clinical outcomes of the study subjects was analyzed using the Chi-square test or Fisher's exact test. Based on these analyses, no statistically significant association was found between the levels of biofilm production (weak, moderate, strong producers) and the clinical outcomes of the subjects (alive, deceased) (p > 0.05).

Table 3. Factors influencing clinical outcomes in the study subjects

Variable	Clinical outcomes		p
	Alive (n = 10)	Deceased (n = 10)	
Median of patient's ade (years)	39,5 (18 - 81)	38 (22 - 79)	0,97
Male sex	4 (30,8%)	9 (69,2%)	0,057
Median of BMI (kg/m^2)	26,4 (20,0 - 33,2)	23,4 (19,4 - 27,7)	0,173
Comorbidities	7 (58,3%)	5 (41,7%)	0,65
Initial admission to intensive care unit	5 (41,7%)	7 (58,3%)	0,65
Median of duration from admission to infection (days)	14 (5 - 43)	14 (3 - 43)	0.93
Median of APACHE II score	9 (3 - 17)	18,5 (9 - 27)	0,004
Inappropriate empirical antibiotic therapy	5 (50%)	5 (50%)	1,000

Analysis of the factors influencing clinical outcomes in the study subjects was performed using the Chi-square test or Fisher's exact test and the Mann-Whitney test. A statistically significant difference was found in the APACHE II score category (p < 0.05). This indicates that the APACHE II score is one of the factors influencing the clinical outcomes of the study subjects.

Table 4. The relationship between biofilm production capacity and antibiotic resistance of gram-negative bacteria

Biofilm production capacity	Resistance phenotypes		p
	Non-MDR n (%)	MDR n (%)	
Weak producer	2 (33,33)	2 (14,3)	0,779
Moderate producer	3 (50)	10 (71,4)	
Strong producer	1 (16,7)	2 (14,3)	

The association between biofilm production ability and the resistance phenotypes of Gram-negative bacteria was analyzed using the Chi-square test or Fisher's exact test. Based on these analyses, no statistically significant association was found between the levels of biofilm production (weak, moderate, strong producers) and resistance phenotypes (non-MDR, MDR) (p > 0.05).

Discussion

This study demonstrated a significant association between the time of specimen collection following empirical antibiotic administration and the level of biofilm production by Gram-negative bacteria (p < 0.05). Antibiotic exposure particularly at sub-inhibitory concentrations is known to induce biofilm formation through several mechanisms, including activation of bacterial stress responses, increased cyclic di-guanosine monophosphate (c-di-GMP) signaling, modulation of quorum sensing, and enhancement of extracellular matrix components such as e-DNA and exopolysaccharides. Hoffman et al. (2003) reported that sub-MIC concentrations of aminoglycosides stimulate biofilm formation in Pseudomonas aeruginosa and Escherichia coli via the arr regulatory gene.16 Similarly, Baidya et al. (2021) showed that prolonged antibiotic exposure promotes biofilm development and exacerbates resistance in Gram-negative pathogens such as P. aeruginosa and Acinetobacter baumannii.9

In this study, no statistically significant association was observed between biofilm production capacity and clinical outcomes (p > 0.05). However, all subjects infected with strong biofilm-producing isolates experienced poor outcomes (deceased). Chukamnerd et al. (2024) similarly found higher mortality in patients with VAP caused by biofilm-producing A. baumannii, although the difference was not statistically significant. Their study showed that biofilm-forming isolates were significantly associated with bacteremia and antimicrobial resistance, both of which, together with high baseline APACHE II scores and inappropriate empirical therapy, contributed to increase in-hospital mortality.18 Biofilm formation represents a complex microbial survival strategy modulated by genetic regulation and the local microenvironment; thus, clinical outcomes are also influenced by host immune responses, antibiotic effectiveness, and other medical complications.9 The absence of a significant association in this study suggests heterogeneity in patient characteristics, variability in host factors, and the possibility that biofilm contributes indirectly rather than directly to mortality.

Among the clinical variables analyzed, the APACHE II score was identified as a significant factor influencing clinical outcomes ($p < 0.05$). This finding highlights the importance of systemic disease severity in determining prognosis among mechanically ventilated pneumonia patients. Consistent with Chukamnerd et al. (2024), higher APACHE II scores were associated with increased in-hospital mortality. As a composite indicator of acute physiological derangement, a high APACHE II score is also linked with an elevated risk of nosocomial infections, delayed recovery, and overall poorer outcomes. These results underscore the importance of comprehensive patient assessment and the utility of APACHE II scoring in guiding timely and targeted interventions in intensive care settings.17,18

This study found no significant association between biofilm production capacity and resistance phenotypes (non-MDR vs. MDR) ($p > 0.05$). This suggests that MDR strains do not necessarily possess superior biofilm-forming ability compared with non-MDR strains. Similar findings were reported by Cepas et al. (2019), who observed no significant relationship between antimicrobial resistance and biofilm strength in Klebsiella pneumoniae and P. aeruginosa.11 Cusumano et al. (2019) also noted that MDR K. pneumoniae isolates were more frequently weak biofilm producers, indicating that this relationship is inconsistent and potentially influenced by culture conditions, strain-specific genetic regulation, and environmental factors.19

Gajdács et al. (2021) reported no significant correlation between biofilm formation and antimicrobial resistance in P. aeruginosa, emphasizing variations related to biofilm testing methodologies, clinical source of isolates, culture conditions, and genetic mutations such as those in lasR or rpoS.20 Chukamnerd et al. (2024) noted a tendency for A. baumannii biofilm producers to exhibit higher antibiotic resistance, though not at a statistically significant level. Biofilm can contribute to secondary resistance through mechanisms involving quorum-sensing activation, regulation of virulence genes, and horizontal gene transfer (HGT) of resistance genes within biofilm communities.18

Biofilm formation and antimicrobial resistance represent distinct but interacting bacterial survival strategies. Genetic resistance provides direct protection against antibiotic action, whereas biofilm offers additional physical and physiological protection that may prolong infection and complicate therapy.11 Plasmid-mediated gene transfer further links these mechanisms, as plasmids often carry both resistance determinants and genes involved in quorum-sensing and biofilm development.21 The absence of a significant association in this study reflects the multifactorial and strain-dependent nature of these mechanisms. Molecular characterization of biofilm- and resistance-related genes may clarify the interplay between these phenotypes and improve diagnostic approaches in biofilm-associated infections.

Conclusion

Mechanically ventilated pneumonia patients in this study were predominantly male with a median age of 39.5 years, BMI 23.9 kg/m^2, frequent comorbidities, early ICU admission, a median post-infection stay of 14 days, median APACHE II score of 13, and mostly received single-agent cephalosporin, with specimens collected a median of 5.5 days after empirical antibiotics. Gram-negative isolates showed 20% weak, 65% moderate, and 15% strong biofilm production, influenced by the timing of specimen collection. Biofilm production was not significantly associated with clinical outcomes, whereas the APACHE II score was a significant predictor. No significant association was also found between biofilm production capacity and Gram-negative resistance phenotype.

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Conflict of interest

The authors declare there is no conflict of interest.

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Authors' contribution

All authors contributed toward data analysis, drafting, and revising the paper and have collectively assumed responsibility for all aspects of the work.

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