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RESEARCH ARTICLE

Clinical isolates from ICU patients show high rates of biofilm production: A descriptive study

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ABSTRACT

Biofilm formation on indwelling devices promotes antimicrobial resistance and complicates management of ICU infections; comprehensive profiling of biofilm-producing pathogens in ICU settings is needed to inform infection control and stewardship. Cross-sectional study (June–September 2023) of clinical isolates from ICU patients with sepsis, pneumonia, urinary tract infection, or wound infection at Sultan Agung Islamic Hospital, Semarang. Sixty-two isolates from sputum (endotracheal aspirates), pus/wound swabs, urine, and blood were identified by standard culture and biochemical methods. Biofilm production was assessed by the 96 well microtiter plate crystal violet assay. All isolates produced biofilm: 50/62 (80.6%) strong, 9/62 (14.5%) moderate, and 3/62 (4.8%) weak. Gram negative organisms predominated: *Klebsiella pneumoniae* 18/62 (29.0%), *Pseudomonas aeruginosa* 17/62 (27.4%), and *Escherichia coli* 10/62 (16.1%). All *P. aeruginosa* isolates (17/17; 100%) exhibited strong biofilm formation across clinical conditions. *K. pneumoniae* was the most frequent cause of pneumonia (15/62; 24.2%), with 13/15 (86.7%) strong biofilm producers. ICU isolates showed a high prevalence of strong biofilm production, primarily among Gram negative pathogens. These findings support the implementation of targeted surveillance, strict device care protocols, and antimicrobial stewardship strategies to address biofilm-associated resistance; larger multicenter longitudinal studies are recommended.

1. Introduction

Biofilms are complex microbial communities that form aggregates and attach to device surfaces, creating a protective environment for the microorganisms they encase (Roy *et al.,* 2018). In the intensive care unit (ICU), extensive use of indwelling medical devices, such as mechanical ventilators, central venous catheters, and intravenous lines, provides ideal substrates for biofilm formation. This encapsulation shields bacteria from host immune responses and antimicrobial agents, substantially contributing to multidrug resistance (MDR) (Deglovic *et al.,* 2022; Romário-Silva *et al.,* 2022). As a result, biofilm-associated infections complicate patient management, prolong hospital stays, increase healthcare costs, and raise morbidity and mortality (Levy *et al.,* 2018; Zhang *et al.,* 2021). Although preventive measures, such as strict oral hygiene and

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scheduled device replacement, are routinely applied, device-associated infections remain a persistent clinical challenge (Fukami *et al.,* 2022; Levy *et al.,* 2018).

Previous studies have documented specific MDR and biofilm-producing pathogens in critical care settings. For example, *Acinetobacter baumannii* has drawn global attention for its high prevalence and extensive resistance in ventilator-associated pneumonia (VAP) (Ibrahim *et al.,* 2021). Other major nosocomial pathogens—including extended-spectrum beta-lactamase (ESBL)– producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Pseudomonas aeruginosa,* and vancomycin-resistant *Enterococcus spp.*—have also been reported to be potent biofilm producers (Oliveira *et al.,* 2020; Dargahi *et al.,* 2022).

Despite this evidence, a gap remains in comprehensive biofilm profiling across the broader spectrum of bacteria responsible for infections in the ICU. Much of the literature focuses on individual species or specific device-associated infections, such as VAP, while comparative data mapping biofilm-forming capacity across diverse clinical isolates and correlating these findings with current MDR patterns remains limited. This study, therefore, aims to characterise the biofilm profile of bacteria causing sepsis, pneumonia, urinary tract infections, and wound infections in the ICU, to inform targeted antimicrobial strategies and improve patient management.

2. Materials and Methods

Study design

This cross-sectional descriptive study was conducted from June to September 2023. All clinical samples submitted to the microbiology laboratory at Sultan Agung Islamic Hospital in Semarang, Indonesia, were included. Sultan Agung is a referral hospital in Semarang city. Eligible patients were ≥18 years old and had received intensive care for at least one day. For each patient, demographic and clinical data were recorded, including age, sex, isolated organism, specimen type, and biofilm-production result.

Microbial identification

Blood cultures were performed when bloodstream infection was suspected. Primary inoculation was done in trypticase soy broth prepared in the laboratory; samples showing growth indicators (hemolysis, gas, or turbidity) were subcultured onto appropriate solid media for further identification. Fastidious organisms were subcultured on chocolate agar, while non-fastidious organisms were subcultured on blood and MacConkey agar. Gram staining was performed on isolated colonies. Initial identification was based on colony morphology and Gram reaction. Gram-positive bacteria were further cultured on mannitol salt agar and blood agar and tested with coagulase, catalase, optochin, bile solubility, PYR, novobiocin, and bile esculin as indicated. Gram-negative bacteria were cultured on eosin methylene blue and MacConkey agar and identified by biochemical tests, including oxidase, catalase, triple sugar iron (TSI), citrate, urease, lysine iron agar (LIA), sulfide–indole–motility (SIM), and indole. All identification followed standard procedures and quality-control strains; equivocal results were repeated (Mahon, 2019).

Sample collection

Clinical specimens for culture included sputum (endotracheal aspirates via a Lukens trap in patients with clinical and radiographic signs of pneumonia), urine, wound swabs/pus, and blood. Specimens were collected using sterile technique and transported to the laboratory in appropriate containers.

Biofilm formation assay

Biofilm production was assessed by the 96-well microtiter plate assay. Isolates were first grown overnight at 37°C on Mueller–Hinton agar. Colonies were suspended in normal saline (0.85% NaCl) and adjusted to 0.5 McFarland (1.5 × 108 CFU/mL). A 10-µL aliquot of each suspension was diluted 1:200 in 190 µL tryptic soy broth (TSB) supplemented with 1% glucose in polystyrene 96-well plates. Plates were incubated at 37°C overnight. Wells were washed three times with phosphate-buffered saline (PBS), fixed with 200 µL methanol, and stained with 200 µL 0.1% crystal violet for 20 minutes. After three additional PBS washes, the bound dye was solubilised in 200 µL of 95% ethanol for 10 minutes. Optical density (OD) was measured at 595 nm using an ELISA plate reader. *Staphylococcus epidermidis* ATCC 35984 and sterile TSB served as positive and negative controls, respectively. Biofilm interpretation followed the criteria of Zhang *et al.* (2016). All assays were performed in triplicate.

Ethics statement

The study protocol was approved by the Research Ethics Committee of Faculty of Medicine, Universitas Islam Sultan Agung. Written informed consent was obtained from participants or from relatives when patients were unable to provide consent.

3. Results

During the study period, 62 clinical samples yielded bacterial growth. Table 1 summarises patient characteristics, specimen types, biofilm-production

Table 1. Distribution of patient characteristics with bacterial isolates.

Characteristics	Total (%)
Sex	
• Male	32 (51,6%)
• Female	30 (48,4%)
Age	
• 18 – 24 years	5 (8,1%)
• 24 – 64 years	12 (19,3%)
• > 64 years	45 (72,6%)
Specimen	
• Sputum	25 (40,3%)
• Pus	17 (27,4%)
• Urine	7 (11,2%)
• Blood	13 (20,9%)
Biofilm Production	
• Negative	0 (0%)
• Weak	3 (4,8%)
• Moderate	9 (14,5%)
• Strong	50 (80,6%)
Biofilm-Producing microorganisms	
• *K. pneumoniae*	18 (29,0%)
• *P. aeruginosa*	17 (27,4%)
• *E. coli*	10 (16,1%)
• *E. cloacae*	4 (6,4%)
• *S. aureus*	4 (6,4%)
• *E. aerogenes*	3 (4,8%)
• *A. baumannii*	2 (3,2%)
• *S. maltophilia*	2 (3,2%)
• *P. mirabilis*	1 (1,6%)
• *MRSA*	1 (1,6%)

categories, and organism distribution. Most isolates came from male patients (n = 32; 51.6%). The majority were from patients aged >64 years (n = 45; 72.6%), followed by 24–64 years (n = 12; 19.3%) and 18–24 years (n = 5; 8.1%). Specimen distribution was sputum 25 (40.3%), pus 17 (27.4%), blood 13 (20.9%), and urine 7 (11.2%). All isolates produced biofilm: 50 (80.6%) were strong producers, 9 (14.5%) moderate, and 3 (4.8%) weak. Distribution of biofilm-producing organisms (n = 62) was: *Klebsiella pneumoniae* 18 (29.0%), *Pseudomonas aeruginosa* 17 (27.4%), *Escherichia coli* 10 (16.1%), *Enterobacter cloacae* 4 (6.4%), *Staphylococcus aureus* 4 (6.4%), *Enterobacter aerogenes* 3 (4.8%), *Acinetobacter baumannii* 2 (3.2%), *Stenotrophomonas maltophilia* 2 (3.2%), *Proteus mirabilis* 1 (1.6%), and Methicillin-resistant *Staphylococcus aureus* (MRSA) 1 (1.6%).

P. aeruginosa produced the strongest biofilms across all its clinical presentations: sepsis (11/11; 100%), ulcers (3/3; 100%), and pneumonia (3/3; 100%). *K. pneumoniae* was the leading cause of pneumonia (15/18; 83.3% of *K. pneumoniae* isolates; overall pneumonia cases n = 15), of which 13/15 (86.7%) were strong biofilm producers and 2/15 (13.3%) were moderate producers. Detailed correlations between Gram-negative species, clinical conditions, and biofilm-strength categories are presented in Table 2. Among Gram-positive isolates, S. aureus (4/4; 100%) produced strong biofilms in pneumonia (3/3; 100%) and ulcer (1/1; 100%) cases. MRSA was isolated from one ulcer case and was a strong biofilm producer (1/1; 100%).

Table 2. Correlation of gram-negative bacterial biofilm production with patient clinical conditions.

Microorganisms (n)	Clinical Condition (n)	Biofilm Production, n (%)			
		Negative	Weak	Moderate	Strong
K. pneumoniae (n = 18)	• Pneumonia (n = 15)	0 (0%)	0 (0%)	2 (13,3%)	13 (86,7%)
	• Ulcer (n = 2)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
	• Urinary tract infection (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
P. aeruginosa (n = 17)	• Sepsis (n = 11)	0 (0%)	0 (0%)	0 (0%)	11 (100%)
	• Ulcer (n = 3)	0 (0%)	0 (0%)	0 (0%)	3 (100%)
	• Pneumonia (n = 3)	0 (0%)	0 (0%)	0 (0%)	3 (100%)
Escherichia coli (n = 10)	• Ulcer (n = 5)	0 (0%)	1 (20,0%)	3 (60,0%)	1 (20,0%)
	• Urinary tract infection (n = 4)	0 (0%)	0 (0%)	2 (50,0%)	2 (50,0%)
	• Pneumonia (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
E. cloacae (n = 4)	• Urinary tract infection (n = 2)	0 (0%)	0 (0%)	2 (100%)	0 (0%)
	• Ulcer (n =1)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
	• Pneumonia (n =1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
E. aerogenes (n = 3)	• Ulcer (n = 2)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
	• Pneumonia (n = 1)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
A. baumannii (n = 2)	Pneumonia (n =2)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
S. maltophilia (n = 2)	• Pneumonia (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
	• Ulcer (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
P. Mirabilis (n = 1)	Ulcer (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)

Table 3. Correlation of Gram-positive bacterial biofilm production with patient clinical condition

Microorganisms (n)	Clinical Condition (n)	Biofilm Production, n (%)			
		Negative	Weak	Moderate	Strong
S. *aureus* (n = 4)	• Pneumonia (n = 3)	0 (0%)	0 (0%)	0 (0%)	3 (100%)
	• Ulcer (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
MRSA (n = 1)	Ulcer (n = 1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)

The full breakdown for Gram-positive organisms is shown in Table 3.

4. Disscussion

The study findings indicate that *Klebsiella pneumoniae* was the most frequent biofilm-producing organism, followed by *Pseudomonas aeruginosa* and *Escherichia coli.* These results are consistent with Gogoi and Sharma (2021), who reported *K. pneumoniae* (39.4%) and *P. aeruginosa* (16.9%) as the predominant ICU biofilm producers. In contrast, Dargahi *et al.* (2022) found *Streptococcus spp.* followed by *K. pneumoniae* as the leading biofilm producer in an ICU in Iran. Differences between studies may reflect variation in specimen types (for example, studies limited to endotracheal aspirates), geographic and epidemiologic factors, patient populations, sample size, and study period.

In our study, *P. aeruginosa* demonstrated uniformly strong biofilm formation (n = 17; 100%). This observation aligns with Khatoon *et al.* (2028), who identified *P. aeruginosa* as a prominent opportunistic pathogen with potent biofilm-forming ability and with other reports documenting high biofilm prevalence among clinical *P. aeruginosa* isolates (Cangui-Panchi *et al.,* 2022). Strong biofilm formation by *P. aeruginosa* is clinically important because biofilms markedly increase the risk of multidrug resistance (MDR). Mechanisms include the extracellular polymeric substance (EPS) matrix that impedes antimicrobial penetration, microenvironmental gradients (hypoxia, nutrient limitation) that induce metabolically dormant persister cells less susceptible to antibiotics, and biofilm-associated virulence factors (e.g., pyocyanin) that support biofilm integrity and resistance (Al-Orphaly *et al.,* 2021). Chimi *et al.* (2024) reported MDR in 65.8% of *P. aeruginosa* isolates and emphasised the role of biofilm production in driving resistance (Chimi *et al.,* 2024).

The predominance of strong, biofilm-forming *P. aeruginosa* in the ICU presents a significant clinical threat, contributing to treatment failure, prolonged mechanical ventilation, increased healthcare costs, and higher morbidity and mortality. These findings support the need for targeted infection-control measures and active surveillance of MDR, biofilm-producing organisms in critical care settings. Practical steps include strict device-care bundles, timely removal or replacement of indwelling devices when possible, enhanced environmental cleaning, and antimicrobial stewardship that considers biofilm-associated resistance.

Future research should include larger, longitudinal multicenter studies to (1) define temporal and geographic trends in biofilm-producing pathogens, (2) investigate in vivo triggers and host–device interactions that promote biofilm formation, and (3) evaluate preventive and anti-biofilm therapeutic strategies (including novel antibiofilm agents and device coatings). Such efforts are essential to inform interventions that reduce biofilm-associated infections and improve outcomes in ICU patients.

5. Conclusions

All bacterial isolates recovered from ICU patients in this study were biofilm producers, most of which exhibited strong biofilm-forming capacity. Gram-negative organisms predominated, with Pseudomonas aeruginosa among the strongest biofilm producers and Klebsiella pneumoniae the most frequent isolate. These findings highlight the need for enhanced infection-control measures and antimicrobial stewardship in the ICU, including targeted surveillance of biofilm-forming pathogens, strict device-care practices, and strategies to prevent and manage biofilm-associated infections.

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Conflict of interest

The authors declare no conflicts of interest.

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