



MICROBIOLOGICAL FEATURES AND MECHANISMS OF ANTIBIOTIC RESISTANCE OF BIOFILM FORMATION IN BACTERIA OF CLINICAL ORIGIN

Lamiya Jeyhun Khalilzada¹, Aygun Samad Suleymanova^{1*}  

Western Caspian University, Department of Natural Sciences, Baku, AZ1072, Azerbaijan

Abstract: The existence of bacteria within biofilms provides high resistance against environmental stress, host immune responses, and antimicrobial agents. In this study, the biofilm formation capacity, architectural organization, and resistance mechanisms of six major clinical pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*) were evaluated through comparative and systematic microbiological analyses. The findings indicate that 66.7% of the analyzed isolates are Gram-negative and 66.7% are facultative anaerobes, with 100% displaying colonization capabilities on medical device surfaces. Extracellular polymeric substances (EPS), extracellular DNA (eDNA), outer membrane vesicles (OMVs), and quorum sensing systems play critical roles in recalcitrance and survival.

Keywords: biofilm, clinical pathogens, extracellular matrix, antibiotic resistance, quorum sensing, nosocomial infections.

*Corresponding author: aygun.suleymanova@wcu.edu.az

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Introduction

One of the most pressing challenges facing microbiology and clinical medicine in the modern era is the resistance mechanisms developed by microorganisms to antibiotics. According to the World Health Organization (WHO), effective treatment of infectious diseases is becoming increasingly complex and poses global epidemiological risks as a result of the spread of antibiotic-resistant bacteria (Percival et al. 2015). Studies show that in natural and hospital environments, microorganisms exist in the form of biofilms — cell communities that live within the polymer matrix secreted by themselves, adhering to surfaces rather than in the free

planktonic form (Donlan & Costerton 2002; Zhang et al. 2024).

The widespread use of catheters, artificial heart valves, orthopedic prostheses, endotracheal tubes, and other invasive devices in medical practice create favorable conditions for the formation of biofilms (Hall-Stoodley et al. 2004; Flemming et al. 2016; Percival et al. 2015). Bacteria located within the biofilm are protected against both immune reactions of the body (phagocytosis, antibodies) and traditional antibiotic therapy, causing recurrent and chronic nosocomial infections (Ciofu & Tolker-Nielsen 2019; Sharma et al. 2019). From this point of



view, the comparative study of the stages of biofilm formation, the biochemical composition of the matrix, and the mechanisms of resistance to antibiotics in bacteria of clinical origin are of theoretical and practical importance (Høiby et al. 2010; Liu et al. 2024).

Materials And Methods

In this research, six main pathogens, which are widespread in clinical practice and can form biofilms, were selected for analysis: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella*

pneumoniae, and *Acinetobacter baumannii*.

The research methodology is based on the methods of systematization of scientific literature data, comparative microbiological analysis, tabulation, and descriptive-statistical generalization. The Gram affiliation, oxygen requirement, primary adhesion structures (whip, pili, fimbriae, adhesins), extracellular matrix components (alginate, PIA, extracellular DNA, capsule), Quorum Sensing systems, and clinical colonization foci of each selected pathogen species were compared, as shown in **Table 1**.

Table 1. Comparison of biofilm-forming characteristics of major pathogens of clinical origin.

Bacteria Type	Gram Type	The Relationship Between Oxygen and Oxygen	Main Adhesion and Matrix Factors	Clinical Source/Significance
<i>Pseudomonas aeruginosa</i>	Gram-negative	Aerobe	Whip, Battery, Alquin Matrix, Quorum Sensing	Lung (cystic fibrosis), burn wounds, respiratory tubes
<i>Staphylococcus aureus</i>	Gram-positive	Facultative anaerobic	Adhesins, amyloid fibers, protein-polysaccharide matrix	Chronic wounds, skin infections, catheters, prostheses
<i>Staphylococcus epidermidis</i>	Gram-positive	Facultative anaerobic	Polysaccharide intercellular adhesin (PIA), surface proteins	Catheters, Artificial Heart Caps, Orthopedic Implants
<i>Escherichia coli</i>	Gram-negative	Facultative anaerobic	Fimbriae, battery, eDNA, amyloid fibers (Curli)	Urinary catheters, recurrent urinary tract infections
<i>Klebsiella pneumoniae</i>	Gram-negative	Facultative anaerobic	Thick capsule polysaccharides, fimbrial adhesion	Respiratory tract, artificial ventilation devices, urinary tract
<i>Acinetobacter baumannii</i>	Gram-negative	Aerobe	Outer membrane proteins, OMV secretion, dry surface tolerance	Resuscitation equipment, wound infections, devices

Results and Discussion

Stages of Biofilm Formation and Matrix Architecture

The process of biofilm formation of clinical bacteria takes place in four main phases: primary (reversible) adhesion, fixation (irreversible binding), maturation,

and dispersion, which is characterized by the formation of microcolonies. In the initial phase, weak electrostatic and hydrophobic interactions with the abiotic surface are established by means of pili and batteries. In the irreversible phase, surface adhesions are activated, and the synthesis of extracellular



polymeric substances (EPS) is activated. In the adult biofilm phase, a complex system of 3D water channels is formed, which ensures the transport of food and oxygen to the inner layers, as well as the excretion of metabolites. During the dispersion phase, some bacteria leave the matrix and return to the planktonic form, colonizing new tissue or implant surfaces.

Up to 90% of the biofilm mass is made up of water, and 50–90% of the dry mass is made up of polysaccharides. Structural

proteins, lipids, extracellular DNA (eDNA), and bivalent cations such as Ca^{2+} and Mg^{2+} within the matrix form ion bridges, ensuring the structure's rheological elasticity and mechanical stability.

Statistical Generalization of Microbiological Indicators

The total percentage distribution of microbiological parameters for clinical pathogens included in the analysis is presented in **Table 2**.

Table 2. Distribution of biofilm pathogens by basic microbiological properties

And then there's the parameter that comes with it	Number of species	Interest Share (%)	Microbiological Significance
Gram-negative pathogens	4 / 6	66.7%	Strong aggregation by outer membrane, capsule, battery, and OMV secretion
Gram-positive pathogens	2 / 6	33.3%	Mechanism of Adhesion Proteins and Polysaccharide Intercellular Adhesion (PIA)
Facultative anaerobic types	4 / 6	66.7%	Metabolic plasticity and survival in deep anoxic layers
Absolute Aerobic Species	2 / 6	33.3%	Respiratory system and intensive development on open biotic/abiotic surfaces
Colonization on the surface of a medical device	6 / 6	100.0%	Universal biofilm potential on catheter, prosthetic and implant surfaces
Polysaccharide/Capsule Matrix Species	5 / 6	83.3%	Ensuring the protective and rheological stability of the EPS matrix
Movement and fimbrial adhesion	4 / 6	66.7%	Acceleration of primary adhesion and strengthening of microcolonies

Mechanisms of Tolerance and Resistance to Antibiotics

The high resistance of bacteria to antimicrobial substances within the biofilm is determined by complex factors interacting with each other:

- Diffusion barrier and electrostatic trap: A dense EPS matrix and negatively charged extracellular DNA (eDNA) bind to positively charged antibiotics (e.g., aminoglycosides) and prevent their free diffusion into the inner

layers.

- Persistent cells and metabolic stagnation: Hypoxia and malnutrition that occur deep in the biofilm move cells into a slow or passive metabolic phase. Drugs such as β -lactames, which target multiplying cells, are unable to affect this persistent population.
- Outer Membrane Vesicles (OMVs): OMVs secreted by Gram-negative pathogens such as *P. aeruginosa* and



A. baumannii inactivate antibiotic molecules by capturing them on the surface before reaching the cell.

- Utilization of Neutrophil Extracellular Traps (NETs): DNA chains released by neutrophils at the infection site are incorporated into the EPS matrix by *S. aureus* and *K. pneumoniae* as mechanical fixtures, further strengthening the biofilm architecture.
- Efflux pumps and Horizontal Gene Transfer (HGT): Efflux pumps, activated by quorum sensing signals, remove antibiotics from the cell. At the same time, the dense location of the cells accelerates the transmission of resistance plasmids by conjugation.

Conclusions and Suggestions

Biofilm formation in bacteria of clinical origin is an important adaptation strategy that allows them to evade drugs and the body's immune control. The study shows that most biofilm-forming pathogens are Gram-negative (66.7%) and facultative anaerobic (66.7%) and have 100% colonization ability on medical implants and catheters. The biofilm matrix acts not only as a mechanical barrier but also as a biochemically active defense system. As practical suggestions, the following are offered:

- Regular microbiological monitoring should be carried out for the early detection of biofilm-forming strains in clinical facilities and intensive care units.
- The use of nanotechnological coatings with antibiofilm properties in the use of invasive medical devices (catheters, implants, prostheses) should be expanded.
- To prevent the adaptation of bacteria to sublethal doses, strict adherence to the protocols for the rational use of antibiotics should be strictly observed.

Conflict of Interest

The authors declare that there is no conflict of interest.

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