Chapter 20: Diagnostic Approaches for Biofilm-Associated Infections*

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Abstract

Biofilms, which are bacterial colonies encased in a self-produced matrix, are central to many persistent infections, particularly in medical devices, dental health, respiratory disorders, and chronic conditions. Their complex structure and behavior present significant diagnostic and therapeutic challenges. Understanding biofilm formation and implementing advanced diagnostic and treatment strategies are essential for improving patient outcomes. Biofilms exhibit antimicrobial resistance through several mechanisms, including the protective extracellular matrix, slow-growing or dormant bacterial states, and increased horizontal gene transfer, which promotes resistance. These factors significantly reduce the efficacy of antibiotics. Additionally, the biofilm's architecture limits immune cell penetration, weakening the host immune response and contributing to the persistence and chronic nature of biofilm-related infections.

Conventional diagnostic techniques, such as culturing and microscopy, are often inadequate for detecting biofilm-associated infections due to the biofilm's complexity and slow growth. Advanced approaches like scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), and molecular methods such as PCR offer improved sensitivity but still face challenges due to biofilm heterogeneity. Emerging technologies, such as real-time imaging, high-throughput sequencing, and novel biomarkers, show promise for enhancing biofilm detection and characterization.

Future advancements should focus on refining these emerging techniques to provide faster, more accurate diagnostics. These innovations could improve the management of chronic biofilm-related infections, especially those associated with medical devices, wounds, and respiratory conditions, ultimately leading to better patient care and outcomes.

Key words: Biofilm; Infectious Diseases; Extracellular polymeric substances; Antimicrobial resistance.

I. Introduction to biofilm-associated infections

Biofilms are communities of microorganisms surrounded by a self-produced matrix of extracellular polymeric substances (EPS), which includes polysaccharides, proteins, and nucleic acids. This matrix provides both structural support and protection for the microbial group. Biofilms can form on both living (e.g., tissues, organs) and non-living (e.g., medical devices, catheters, prosthetics) surfaces. The ability of microorganisms to form biofilms is crucial for their survival within the human body and their resistance to antimicrobial treatments. Biofilms are associated with many chronic and persistent infections, making them a significant issue in clinical and medical research resulting in over 80% of bacterial infections [1][2][3][4]. The key characteristics of bacterial biofilms are primarily linked to their ability to protect bacterial cells and confer resistance to various threats. Bacterial biofilms typically exhibit resistance to both the host immune response and antibiotic treatments. Health-related concerns are escalating due to the potential of biofilms to cause infections, both device-associated and non-device-associated. Dental plaque, a prevalent biofilm, can result in conditions such as gingivitis, periodontitis, and dental caries. Bacteria from the oral cavity may enter the bloodstream, potentially impacting the cardiovascular system and promoting the development of atherosclerotic plaques in the coronary arteries. The inflammatory response triggered by periodontal infections exacerbates plaque accumulation, contributing to edema in the coronary arteries. Research indicates that individuals with periodontal disease are approximately twice as likely to develop coronary artery-related conditions [3][5][6]. Biofilm-associated infections present a significant diagnostic challenge due to the sessile lifestyle of microorganisms, reduced metabolic activity, and frequent failure of standard culture techniques. These infections are commonly linked to chronic wounds, implanted medical devices, and recurrent infections, often exhibiting resistance to both antimicrobial therapy and immune clearance. As such, timely and accurate diagnosis requires a multifaceted approach that integrates clinical, imaging, microbiological, and molecular data. Figure 1 provides an overview of the principal diagnostic strategies currently employed to detect and characterize biofilm-related infections.

Figure 1Overview of diagnostic strategies for biofilm-associated infections

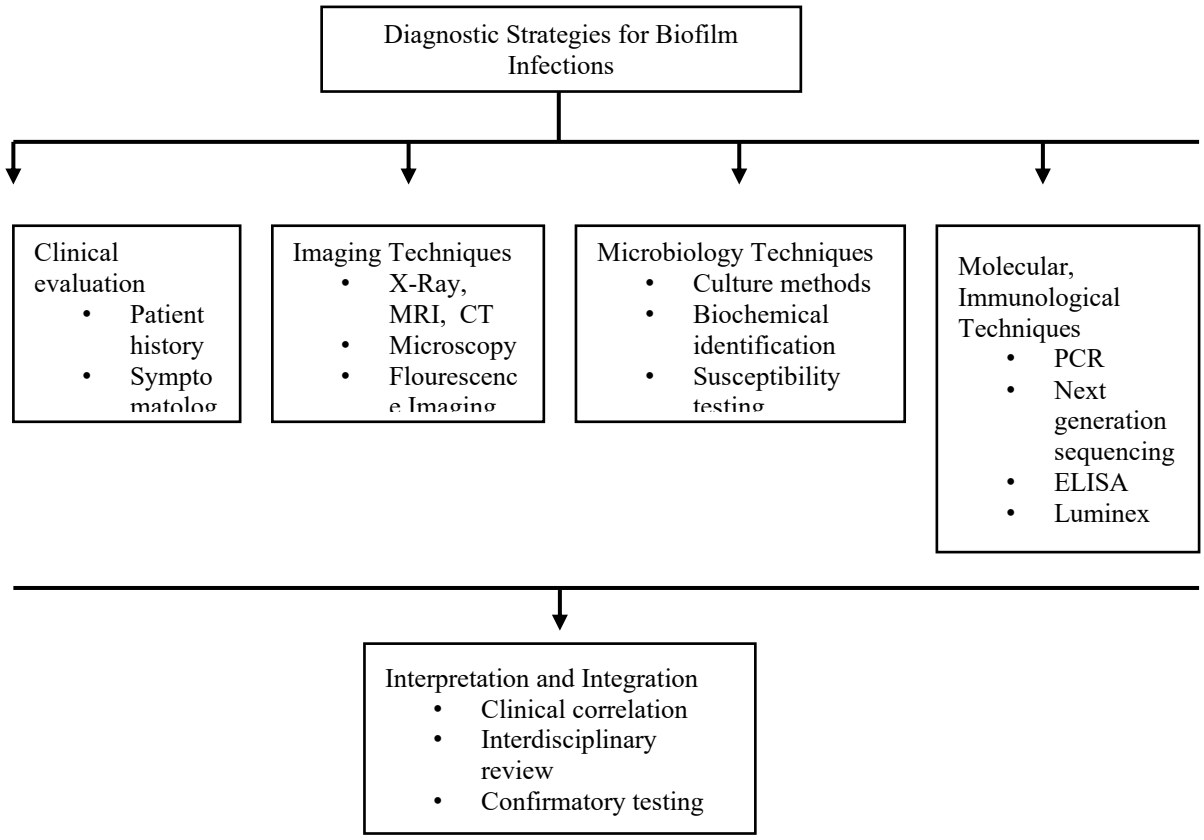

Figure 1 shows an overview of diagnostic strategies for biofilm-associated infections. Diagnosis integrates clinical evaluation (patient history, symptoms, risk factors) with imaging (CT, FM) to identify infection sites. Microbiological methods include standard culture, use of biofilm-enrichment media, and biochemical testing. Molecular and immunological tools (PCR, NGS, ELISA, Luminex) improve sensitivity for detecting low concentration or non-culturable pathogens. Integrated data interpretation is essential for accurate diagnosis and treatment planning. Abbreviations: CT – computed tomography; FM – fluorescence microscopy; PCR –polymerase chain reaction; NGS – next-generation sequencing; ELISA – enzyme-linked immunosorbent assay.

II. Significance of biofilms in infections

Biofilm-associated infections pose a significant challenge in healthcare. Once microorganisms adhere to surfaces and establish biofilms, they exhibit enhanced resistance to antibiotics, host immune responses, and other external factors. This increased resistance is mediated by several mechanisms, including the limited penetration of antimicrobial agents through the biofilm matrix, the altered metabolic states of the microorganisms, and the protective properties of the extracellular polymeric substances (EPS) layer. Biofilm-associated infections are commonly observed in various clinical scenarios, including chronic wounds, urinary tract infections, osteomyelitis, and infections related to indwelling medical devices such as catheters and prosthetic joints. Diagnosing these infections is particularly challenging, as standard culturing methods often fail to detect biofilm-forming bacteria, and the biofilm architecture enables evasion of the host immune system [5].

Biofilms exhibit distinct characteristics that differentiate them from planktonic bacteria, particularly in the context of persistent infections. These adaptations have profound therapeutic implications, including heightened antibiotic resistance, sustained inflammation, tissue damage, and diagnostic difficulties. One of the most notable features of biofilms is their ability to confer antibiotic resistance. Within the protective biofilm matrix, bacteria are shielded from the action of antibiotics, rendering them significantly more resistant to treatment. In addition to antibiotic resistance, biofilms also enhance bacterial resistance to host immune defenses and other therapeutic interventions. This underscores the importance of personalized treatment strategies and highlights the critical need for ongoing research focused on understanding and addressing the complexities of biofilm-associated infections. Chronic infections associated with biofilms are particularly challenging to diagnose. Traditional diagnostic methods, which rely on detecting bacteria in bodily fluids or tissues, often fail to identify biofilm-forming microorganisms, resulting in false-negative outcomes. Furthermore, biofilms play a central role in the development of antibiotic resistance, as their structure creates a barrier that prevents the effective penetration and action of antimicrobial agents, thereby diminishing the efficacy of treatment strategies [7–11].

Bacteria within biofilms typically exhibit slower growth rates and adopt unique metabolic states, which decrease their susceptibility to antibiotics targeting rapidly dividing cells. These difficulties are further exacerbated by the diverse microenvironments within biofilms and the potential for genetic changes that enhance antibiotic resistance. Biofilms are frequently found on medical implants and devices, such as catheters and prosthetic joints, where they present significant challenges in clinical practice. These chronic, biofilm-associated infections are particularly challenging to treat. Beyond their involvement in medical device-related infections, biofilms are also implicated in a variety of chronic conditions, including gastrointestinal, respiratory, urinary tract infections, and chronic otitis media. This chapter explores the multifaceted challenges posed by biofilms in chronic infections, with a focus on their role in antibiotic resistance and their broader impact in different medical contexts [12, 13].

III. Biofilm formation: process and stages

Biofilm formation is a complex, multi-step process that begins with microbial attachment to a surface. This attachment is followed by the secretion of EPS, creating the initial stages of biofilm development. Over time, the biofilm matures, and its structural integrity is enhanced, leading to the formation of water channels and microcolonies that allow the bacteria to communicate through quorum sensing.

The development of a biofilm can be divided into several stages:

a. *Initial Attachment:* Microorganisms adhere to a surface through weak van der Waals forces, followed by more stable attachment mediated by surface molecules like pili and adhesins. The process begins when free-floating (planktonic) microorganisms encounter a surface, such as tissues, medical devices, or environmental surfaces. These microorganisms use various structures, such as pili, flagella, and surface proteins, to adhere to the surface. This attachment is often weak and reversible at this stage. After microorganisms adhere to the surface, they begin synthesizing EPS, which consist of polysaccharides, proteins, and nucleic acids. The formation of EPS enhances the attachment, rendering it irreversible. At this stage, the microorganisms are securely anchored to the surface, and the biofilm formation process progresses[14] [15].

b. *Microcolony formation and Maturation:* Once attached, the organisms secrete EPS, forming a protective barrier that supports microbial growth. As they multiply, microcolonies of bacterial cells develop within the EPS matrix, creating a favorable environment for further growth and division. The EPS also shields the microorganisms from environmental stressors, such as pH fluctuations, temperature changes, and antimicrobial agents [16].

c. *Mature Biofilm:* The biofilm matures with distinct microcolonies and water channels that enable nutrient exchange. As the microcolonies expand, the biofilm develops a three-dimensional structure with channels for nutrient and waste exchange. The microbial community becomes heterogeneous, with species occupying different regions depending on nutrient availability and environmental conditions. The EPS matrix also protects the cells from immune system attacks and antibiotics [16].

d. *Dispersion:* In the final stage, some of the microorganisms leave the biofilm to colonize new areas. Each stage of biofilm formation presents unique challenges for diagnosis, and identifying biofilms early in their development can improve clinical outcomes. Once the biofilm reaches a certain size and density, some cells will detach from the surface and disperse into the surrounding environment. These planktonic cells can then colonize new surfaces, initiating the formation of new biofilms. Dispersion is a critical step in the biofilm lifecycle, allowing the microorganisms to spread and potentially cause infections in new areas. Throughout this process, the biofilm matrix provides structural integrity, facilitates communication between cells (via quorum sensing), and protects the microorganisms from external threats, making biofilms highly resistant to antimicrobial treatments and immune system clearance [16] [17]. In summary, Biofilm formation in *Pseudomonas aeruginosa* as an example, occurs in five distinct stages: reversible attachment, irreversible attachment, maturation phase 1, maturation phase 2, and dispersion [18, 19].

Figure 2: Stages of biofilm formation.

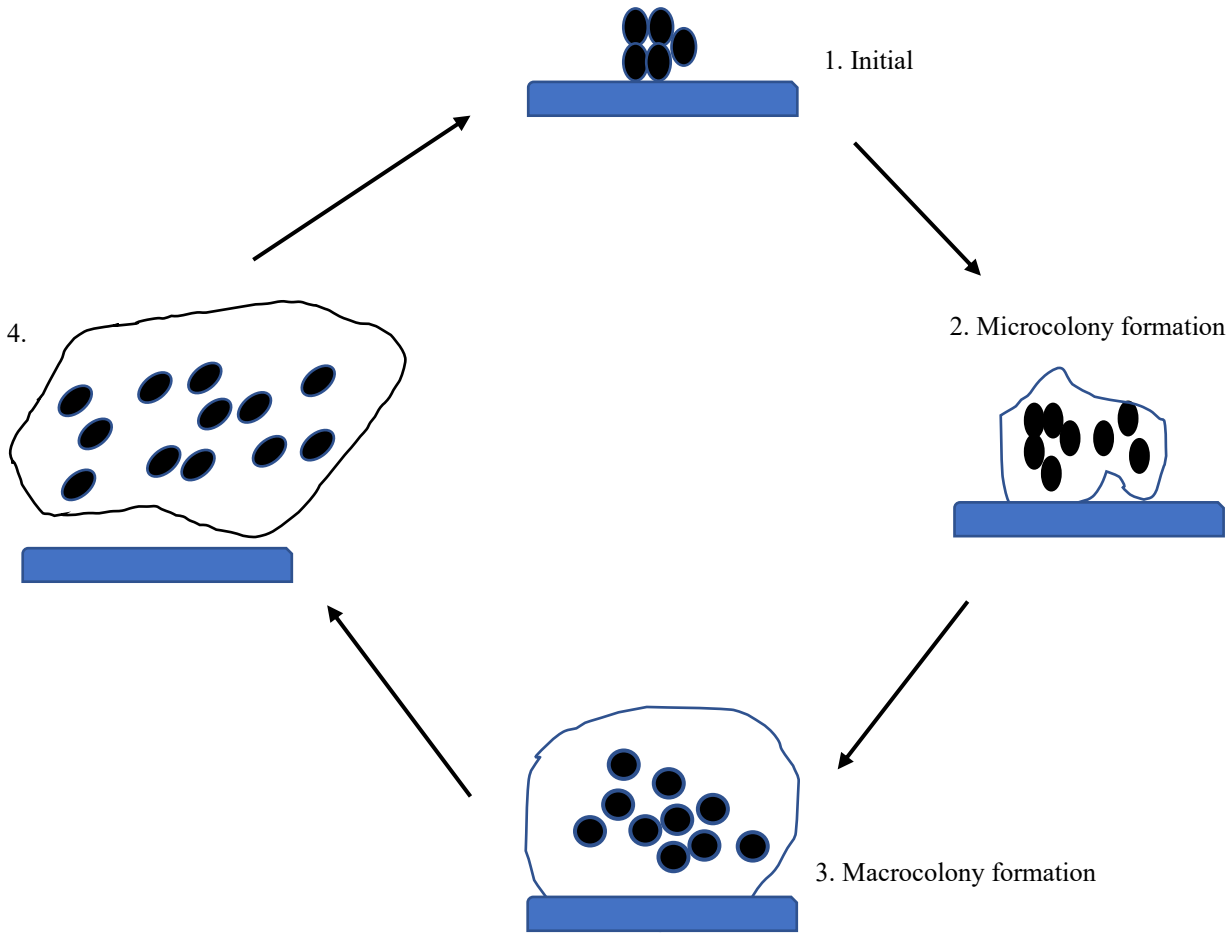

Figure 2, this figure depicts the key stages of biofilm development in *bacteria*: 1. Reversible Attachment: Planktonic cells initially adhere to a surface via weak forces using structures like pili and flagella. In irreversible attachment, EPS production stabilizes adhesion, anchoring cells to the surface. 2. Microcolony Formation: Bacteria proliferate within the EPS matrix, forming microcolonies and enhancing protection. 3. Macrocolony formation and maturation: The biofilm develops a 3D structure with water channels for nutrient exchange and cell signaling via quorum sensing. 4. Dispersion: Some cells detach to colonize new surfaces, promoting biofilm spread and persistence. Each stage enhances biofilm resilience and complicates treatment due to increased resistance to antibiotics and immune responses.

IV. Biofilm pathophysiology

Biofilms are complex, dynamic systems that evolve in response to their surroundings, using a variety of mechanisms to coordinate their formation under different conditions. The process of biofilm formation is carefully regulated by a variety of factors, including the bacteria's physiological state, environmental changes, and the interactions within bacterial colonies. Advances in research have allowed scientists to study bacterial activity at the cellular level, which helps in understanding how biofilms form and how they can be treated more effectively. By activating specific pathways that regulate biofilm formation, bacteria can ensure that they do not form biofilms in unfavorable conditions [17, 20] [21].

Biofilm formation is regulated by several factors, some of which are linked to two-component signaling pathways. These signaling systems help bacteria respond to changes in their environment, ensuring that biofilm formation occurs under optimal conditions. The formation process itself can be broken down into stages. The first stage is the initial adhesion of bacteria to a surface. This starts with reversible adhesion, where bacteria loosely attach to surfaces through non-specific forces such as van der Waals forces, Brownian motion, and electrostatic interactions. Over time, these initial attachments become irreversible, as bacteria strengthen their hold on the surface through various mechanisms, eventually forming a complex three-dimensional biofilm structure [22] [23].

When bacteria encounter a surface, they can sense the contact and adjust their gene expression to promote stable adhesion. The adhesion of bacterial cells to surfaces is often facilitated by specific proteins, known as adhesins, which allow bacteria to stick to host tissues during infection. These adhesins include proteins like fibronectin-binding proteins (FnBPs) and fibrinogen-binding clumping factors (Clfs), which help the bacteria interact with proteins found on the surface of tissues. Once bacteria are within a biofilm, they receive environmental signals that allow them to adjust and colonize the surface more effectively, depending on their

preferences for certain environments. Under conditions where nutrients are either plentiful or scarce, bacteria tend to remain in a planktonic (free-floating) state, as this gives them more access to nutrients and new areas to colonize. However, in the case of medical implants, the competition for surface attachment between bacteria and host cells is critical for the success of the implant. When bacteria (in their planktonic state) are the first to adhere to an implant surface, the body's natural immune defenses are often not strong enough to prevent the biofilm formation. Therefore, the design of the implant itself plays a key role in whether bacterial infections will occur [23] [24] [25].

Bacteria have specialized structures like flagella and pili that help them move in their planktonic state. The flagella, which are much longer than the bacteria themselves, act like motors that propel the bacteria toward surfaces, such as implants. Once the bacteria sense that the environment is suitable for growth, the flagella can anchor the bacteria to the surface. However, if the conditions are not favorable, the bacteria will be pushed away to search for a better habitat. Some bacteria, like *Staphylococcus aureus*, lack flagella and instead rely on pili and other signaling mechanisms to help them attach to surfaces. Shear stress—caused by the movement of fluids—also plays a role in the initial adherence of bacteria to surfaces. Changes in fluid flow can influence how well bacteria stick to surfaces. For example, increased shear stress can lead to the expression of specific substances like polysaccharide intercellular adhesin (PIA), which helps bacteria better adhere to surfaces under such stress [26]. Lectins, another type of protein, also help to promote adhesion and stabilize the early stages of biofilm formation. Surfactants, which reduce surface tension, play a crucial role in helping bacteria adhere to surfaces and form micro-colonies that eventually mature into fully developed biofilms [25][27][28]. Additionally, certain proteins on the bacteria's cell wall, known as cell wall anchoring (CWA) proteins, assist in the binding of bacteria to surface matrices, further supporting the formation and stability of the biofilm. These complex processes enable bacteria to create and maintain biofilms on surfaces, making them much harder to treat or eliminate once they have formed. Understanding these processes is vital for developing strategies to prevent or treat biofilm-related infections, especially in medical devices and implants [28][29] [30].

Biofilm pathophysiology is driven by the unique structural and functional characteristics of biofilms. A biofilm consists of a dense aggregation of microorganisms, often bacteria, embedded in a self-produced extracellular matrix made up of polysaccharides, proteins, and nucleic acids. This matrix provides both structural stability and protection to the microbial community. The biofilm's three-dimensional architecture forms distinct microcolonies, separated by channels that allow for the flow of nutrients and waste products, facilitating the survival of bacteria in a variety of environmental conditions. Functionally, biofilms are highly dynamic systems that exhibit coordinated behavior through quorum sensing, a process by which bacteria communicate to regulate gene expression, virulence, and matrix production. This coordination allows biofilm bacteria to respond adaptively to external changes, such as nutrient availability or stress, making them highly resilient to environmental fluctuations. The structural design of biofilms contributes to their pathogenicity. The matrix acts as a physical barrier, limiting the penetration of antimicrobial agents and immune cells, while the spatial arrangement of bacteria creates microenvironments with varied oxygen, pH, and nutrient gradients. These features enhance bacterial survival, promote resistance to antibiotics, and complicate host immune responses, making biofilm-associated infections chronic and difficult to treat [31].

Antibiotic resistance in biofilms arises through several mechanisms that considerably diminish the efficacy of antimicrobial therapies. A primary factor is the extracellular matrix (ECM), which forms a physical barrier that restricts the penetration of antibiotics into the biofilm. This matrix, composed of polysaccharides, proteins, and nucleic acids, creates a dense protective shield around the bacterial cells, hindering the diffusion of antimicrobial agents. Moreover, bacteria within biofilms frequently enter a slow-growing or dormant state, further contributing to resistance. Antibiotics that target proliferating cells, such as beta-lactams or aminoglycosides, are less effective against these dormant microorganisms, as they are not actively dividing. This metabolic inactivity plays a key role in the bacteria's ability to withstand antibiotic treatment. Additionally, biofilms facilitate the transfer of genetic material, promoting the spread of antibiotic resistance genes between bacterial cells. Through horizontal gene transfer mechanisms like conjugation, transformation, and transduction, biofilm communities can rapidly disseminate resistance traits, enhancing their survival and persistence in the presence of antimicrobial agents [32, 33].

The host immune response to biofilms is significantly hindered by the unique structural and functional properties of biofilms, making it difficult for the immune system to effectively detect and eliminate biofilm-associated bacteria. The ECM that encases biofilm bacteria serves as a physical barrier, impeding the access of immune cells such as macrophages and neutrophils. This protective layer also obstructs the penetration of antibodies and antimicrobial peptides, which are key components of the innate immune system.

Furthermore, the bacteria within biofilms often exhibit altered phenotypic states, such as metabolic dormancy or slow growth, which contribute to their resistance to immune-mediated killing. In these dormant states, biofilm bacteria are less susceptible to reactive oxygen species (ROS) and other immune effector mechanisms that typically target actively dividing cells. Biofilm-associated bacteria also employ various strategies to evade immune detection. For example, some biofilm-forming pathogens can modulate host signaling pathways, suppressing inflammatory responses or interfering with immune cell recruitment. Additionally, the biofilm matrix can trap immune cells, preventing their effective action against bacteria. As a result, biofilm-related infections often become chronic and are associated with persistent inflammation, tissue damage, and poor clinical outcomes, making them difficult to resolve with standard immune defenses alone [1][33][3].

V. Traditional diagnostic approaches for biofilm-associated infections.

Traditional diagnostic methods for biofilm-associated infections struggle due to the unique nature of biofilms. The extracellular matrix surrounding biofilm bacteria impedes effective detection using conventional techniques like culturing and microscopy. Biofilms often fail to grow as efficiently as free-floating bacteria, leading to false negatives in standard culturing methods. Additionally, the altered metabolic states of biofilm bacteria make them less susceptible to laboratory conditions. While

microscopic techniques such as scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) can visualize biofilms, they are time-consuming and require specialized equipment, and may not fully reflect the complexity of biofilms in vivo [34]. Molecular methods like PCR offer increased sensitivity but are still limited by the heterogeneous structure of biofilms, which makes detecting all species within them challenging. As a result, there is a growing need for advanced diagnostic approaches, including real-time imaging, biomarkers, and high-throughput sequencing, to improve biofilm detection and characterization in clinical environments [35, 36].

The absence of biofilm-specific diagnostic methods poses significant challenges in detecting and treating biofilm-associated infections. Biofilms, consisting of microbial communities within an extracellular matrix, display unique structural and physiological properties that differ from planktonic bacteria. Conventional techniques, such as culturing and microscopy, fail to adequately detect biofilms, as their bacteria grow slowly or enter dormant states, resulting in false negatives. The three-dimensional architecture and heterogeneity of biofilms complicate detection, with methods like SEM and PCR facing limitations in capturing their full complexity [35–38]. This highlights the urgent need for specialized diagnostic tools to improve detection and treatment outcomes

VI. Molecular approaches

Nucleic acids, particularly DNA, play a crucial role in the formation of biofilms, especially during the early stages. However, DNA is just one component of the complex biofilm matrix that evolves over time and is concentrated in specific areas rather than being uniformly distributed. Polymerase chain reaction (PCR) and quantitative PCR (qPCR)-based assays are valuable tools for detecting biofilm-associated infections due to their sensitivity and specificity. These molecular techniques amplify and quantify bacterial DNA, enabling the identification of biofilm-forming bacteria even in low concentrations [38, 39].
PCR works by amplifying specific regions of bacterial DNA, making it easier to detect pathogens that may be present in biofilms, which often exhibit low growth rates and are difficult to detect with conventional culturing methods. PCR assays can target unique genetic markers associated with biofilm formation, such as genes involved in the synthesis of extracellular matrix components or adhesion factors, allowing for the detection of biofilm-associated bacteria at an early stage [39] [38].

qPCR, or quantitative PCR, extends the capabilities of traditional PCR by quantifying the amount of target DNA in a sample. It measures the DNA concentration in real-time during the amplification process, providing both qualitative and quantitative data. This allows for not only the identification of biofilm-forming bacteria but also an estimation of their relative abundance in a sample. qPCR is particularly useful in assessing biofilm biomass or monitoring the dynamics of biofilm formation in different conditions, making it valuable for both clinical diagnosis and research. One of the key advantages of PCR and qPCR-based assays for biofilm-associated infections is their ability to detect bacterial DNA even from non-culturable or slow-growing bacteria embedded within the biofilm matrix, which are often missed by traditional diagnostic methods. However, challenges exist, including the complexity of biofilm communities, where multiple bacterial species may coexist, requiring the development of multiplex PCR assays that can simultaneously detect multiple pathogens. qPCR is a highly sensitive molecular technique used to quantify specific DNA or RNA in a sample through DNA/RNA extraction, PCR amplification with specific primers, and real-time fluorescence detection. While it provides quantitative data and supports high-throughput analysis, it can be expensive and prone to contamination. Propidium monoazide (PMA) is a molecule that selectively binds to extracellular DNA from cells with compromised membranes, preventing its amplification during qPCR by forming a stable covalent bond when exposed to visible light [40] [41–43]. Overall, PCR and qPCR-based assays offer high sensitivity and specificity for detecting biofilm-related infections, providing valuable information for diagnosis, monitoring infection progression, and assessing the effectiveness of antimicrobial treatments. The integration of these techniques with other diagnostic methods will further enhance the ability to manage biofilm-associated infections in clinical settings [19, 30, 38, 39].

VII. Metagenomic approaches

Metagenomic approaches have emerged as powerful tools for detecting and characterizing biofilms, offering a more comprehensive and accurate method for studying complex microbial communities [44]. Unlike traditional culture-based techniques, which rely on the growth of individual species, metagenomics enables the simultaneous analysis of all microbial DNA present in a sample, allowing for the identification of both culturable and non-culturable organisms within biofilms. By extracting total DNA from biofilm samples, sequencing technologies such as next-generation sequencing (NGS) provide insights into the microbial diversity, composition, and functional genes involved in biofilm formation and persistence. This approach allows for the detection of multiple bacterial species, including those with antibiotic resistance genes, which are often difficult to identify using conventional methods [44].

Additionally, metagenomic sequencing can provide information on the functional pathways and metabolic processes occurring within the biofilm, helping to elucidate the interactions between microbes and their environment. One of the key advantages of metagenomics is its ability to detect microbial communities in their natural state, without the need for prior cultivation or enrichment, which is particularly valuable for studying complex, multispecies biofilms [45]. Despite its advantages, metagenomic approaches are not without challenges, including high costs, the need for extensive computational resources, and the complexity of data analysis, as the vast amount of sequencing data requires sophisticated bioinformatic tools for interpretation. However, as sequencing technologies advance and costs decrease, metagenomics holds significant promise for improving the detection, diagnosis, and management of biofilm-related infections, particularly in clinical settings where biofilm-associated pathogens are prevalent.

III. MALDI-TOF MS biofilm structure determination

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a powerful analytical technique commonly used to determine the molecular composition of biofilms. This method is particularly effective for analyzing large biomolecules, including proteins, peptides, nucleic acids, and complex organic compounds, which are crucial for understanding the structure and function of biofilms. The MALDI-TOF MS process begins by co-crystallizing the sample with a matrix on a metal target. The matrix absorbs energy when exposed to a laser, leading to the ionization of the sample. This ionization generates positively charged ions, which are then accelerated toward a detector. The time it takes for these ions to reach the detector is measured and used to create mass spectra, which represent the mass-to-charge ratios of the ions. These spectra provide detailed information about the molecular composition of the sample, allowing for precise analysis of its components. The technique of MALDI-TOF MS has become increasingly popular in clinical laboratories, especially for the identification of microorganisms such as bacteria and fungi. In the context of biofilm research, MALDI-TOF MS is particularly valuable due to its ability to analyze complex mixtures of biomolecules that are often present in biofilms. The method compares the protein profiles of microorganisms in the sample with an extensive database of known species' mass spectra. By matching the profiles, researchers can accurately identify microorganisms down to the genus, species, and even subspecies levels. This capability is essential for understanding the microbial diversity and structure within biofilms, which can be highly complex and resistant to conventional diagnostic techniques.

Furthermore, the precision and speed of MALDI-TOF MS make it an ideal tool for routine diagnostics in clinical settings. The ability to rapidly identify and characterize microorganisms at various taxonomic levels enhances our understanding of microbial communities, especially in the context of biofilm-related infections. Biofilms, which consist of microorganisms encased in a self-produced extracellular matrix, pose significant challenges to both treatment and detection. MALDI-TOF MS offers a more efficient and accurate approach compared to traditional methods like culturing, as it provides rapid identification of microorganisms that may be difficult to culture or may exist in a dormant state within biofilms. This efficiency is crucial in clinical microbiology, where timely identification of pathogens can significantly impact treatment decisions. In addition to identifying microorganisms, MALDI-TOF MS can also provide insights into the molecular components of biofilms, such as proteins involved in biofilm formation, adhesion, and resistance mechanisms. Understanding these components is vital for developing targeted therapies to disrupt biofilm formation or enhance the effectiveness of existing antimicrobial treatments [46][47][48, 49]. By utilizing MALDI-TOF MS, researchers and clinicians can gain a deeper understanding of the molecular intricacies of biofilms, facilitating the development of more effective strategies to combat biofilm-associated infections.

Overall, MALDI-TOF MS is a highly effective and versatile tool for biofilm analysis, offering advantages in speed, accuracy, and sensitivity. It plays a critical role in both basic research and clinical diagnostics, providing valuable information for the study and treatment of biofilm-related diseases.

IX. Biofilm-specific biomarkers

Biofilm-specific biomarkers, such as exopolysaccharides and quorum sensing molecules, are critical for the detection, characterization, and study of biofilms. Exopolysaccharides (EPS) are key components of the extracellular matrix that surrounds biofilm-forming microorganisms. They provide structural integrity, protection against environmental stressors, and contribute to the adhesive properties that allow bacteria to adhere to surfaces and form biofilms. EPS, which are typically composed of polysaccharides, proteins, and nucleic acids, are synthesized and secreted by bacteria within biofilms, and their composition varies depending on the microbial species and environmental conditions. Detection of EPS can serve as an important indicator of biofilm presence, providing a means for diagnosing biofilm-associated infections and distinguishing them from planktonic bacterial infections [15, 31].

Quorum sensing molecules, including autoinducers, are signaling compounds used by bacteria to coordinate gene expression and behavior within a biofilm. Quorum sensing enables bacteria to sense their population density and regulate various physiological processes, such as virulence factor production, biofilm formation, and antibiotic resistance. These molecules, which can be classified into different groups based on their chemical structure and mode of action (e.g., acyl-homoserine lactones in Gram-negative bacteria), play a pivotal role in the establishment and maintenance of biofilm communities. Detection of quorum sensing molecules can provide valuable insights into biofilm development and the microbial interactions occurring within a biofilm. Furthermore, targeting quorum sensing pathways holds therapeutic potential, as disrupting quorum sensing may reduce biofilm formation and increase the efficacy of antimicrobial agents [24, 50]. Both exopolysaccharides and quorum sensing molecules represent promising biofilm-specific biomarkers that could be utilized in advanced diagnostic techniques to better detect, monitor, and treat biofilm-associated infections. These biomarkers offer the possibility of more accurate, non-culture-based methods for biofilm detection, which is critical for managing chronic and persistent infections that are difficult to treat with conventional approaches.

X. Antibiotic susceptibility testing in biofilms

Antibiotic susceptibility testing (AST) in biofilms presents significant challenges due to the unique characteristics of biofilm-associated bacteria. Biofilms are highly resistant to antibiotics compared to planktonic (free-floating) bacteria, primarily due to factors such as the physical barrier provided by the extracellular matrix, reduced antibiotic penetration, altered metabolic states of biofilm bacteria, and enhanced gene exchange within the biofilm community. As a result, traditional AST methods, such as broth dilution or disk diffusion, often fail to accurately reflect the true antibiotic susceptibility of biofilm-associated bacteria. These conventional methods typically test bacteria in their planktonic state, not accounting for the protective and adaptive mechanisms within the biofilm. Consequently, biofilm bacteria may appear susceptible to antibiotics in standard assays, leading to misleading results and ineffective treatment strategies [51].

To address these challenges, specialized methods have been developed to assess antibiotic susceptibility in biofilms. Common approaches include the use of biofilm-specific growth models, such as the microtiter plate assay or the Calgary biofilm device, where biofilms are formed on solid or semi-solid surfaces and exposed to antibiotics. Other methods, like the XTT assay [52] and confocal laser scanning microscopy (CLSM), allow for real-time monitoring of biofilm growth and viability under antibiotic treatment. Despite these advancements, AST in biofilms remains complex, as different biofilms exhibit varying degrees of resistance depending on factors such as the bacterial species, biofilm maturity, and environmental conditions. Antibiotic resistance in biofilms can make treating biofilm-related infections more difficult. A recent study used metagenomics to detect resistance factors in both culturable and unculturable oral bacteria [53]. Further research into standardized biofilm-specific AST protocols is necessary to enhance the accuracy and predictability of antibiotic treatment in biofilm-associated infections.

Standard susceptibility testing, such as disk diffusion, is often inadequate for assessing antibiotic resistance in biofilms. Traditional methods primarily evaluate planktonic bacteria, failing to account for the protective extracellular matrix and altered metabolic states within biofilms. As a result, biofilm-associated bacteria may appear susceptible to antibiotics in these assays, leading to inaccurate results. Specialized testing methods, like microtiter plate assays and biofilm-specific models, are needed to better reflect the true antibiotic resistance of biofilm bacteria.

Biofilm-associated bacteria exhibit distinct resistance mechanisms that make them significantly more resilient to antimicrobial agents compared to planktonic counterparts. One of the primary factors is the extracellular matrix (ECM), a complex network of exopolysaccharides, proteins, and nucleic acids, which acts as a physical barrier, limiting the penetration of antibiotics and immune cells [53]. This matrix not only impedes drug diffusion but also protects bacteria from host immune responses. Additionally, bacteria within biofilms often adopt slow-growing or dormant states, rendering them less susceptible to antibiotics that target actively dividing cells, such as beta-lactams or aminoglycosides. Furthermore, biofilms enhance horizontal gene transfer, allowing for the rapid exchange of antibiotic resistance genes among microbial populations, contributing to the spread of resistance within the biofilm community. The heterogeneous microenvironments within biofilms, characterized by variations in oxygen, pH, and nutrient availability, further complicate treatment, as different subpopulations of bacteria may exhibit varying susceptibilities to antibiotics [38, 39]. Quorum sensing, a communication system that regulates gene expression based on bacterial density, also plays a critical role in biofilm resistance. By coordinating gene expression related to virulence, antibiotic resistance, and matrix production, quorum sensing helps biofilm communities adapt to hostile environments and enhance their survival. Collectively, these biofilm-specific resistance mechanisms significantly hinder the effectiveness of standard antimicrobial therapies, leading to chronic and recalcitrant infections.

The Minimum Biofilm Eradication Concentration (MBEC) assay is a method used to evaluate biofilm resistance to antimicrobial agents. It involves growing biofilms on specially designed pegs and exposing them to varying concentrations of antibiotics. After treatment, the biofilms are assessed for survival, and the minimum concentration required to eradicate the biofilm is determined. This assay provides a more accurate representation of biofilm resistance than traditional methods, as it mimics the biofilm's complex structure and its inherent resistance mechanisms to antimicrobial agents [54]. Modified microdilution methods for biofilms assess antibiotic susceptibility by exposing biofilm-forming bacteria to varying concentrations of antimicrobial agents in a controlled environment. These methods, often adapted from standard broth microdilution techniques, include additional steps such as pre-incubation to allow biofilm formation. They enable the evaluation of the antimicrobial activity against biofilms, providing more accurate data for assessing the effectiveness of treatments against biofilm-associated infections, which exhibit heightened resistance compared to planktonic bacteria. Recent advancements in antimicrobial testing for biofilms focus on improving accuracy and replicating the complex biofilm environment. Techniques such as high-throughput screening, microfluidic systems, and advanced imaging technologies offer more precise evaluation of biofilm growth and resistance [54]. Additionally, the development of novel assays like the MBEC assay and the use of biofilm-specific biomarkers enhance the assessment of antimicrobial efficacy. These innovations facilitate better understanding of biofilm behavior, enabling the development of more effective therapeutic strategies to combat biofilm-associated infections.

XI. Challenges and future directions in diagnostic approaches

Diagnostic approaches for biofilm-associated infections face significant challenges due to the unique structural and physiological properties of biofilms. Traditional methods, such as culturing and microscopy, often fail to detect biofilms, as they are slow-growing and encapsulated in a protective extracellular matrix. Advanced techniques like SEM, CLSM, and PCR offer improved sensitivity but are limited by time requirements, equipment needs, and biofilm complexity [46]. Future directions should focus on developing rapid, non-invasive diagnostic methods, real-time imaging technologies, and high-throughput sequencing. These innovations will enhance biofilm detection, improving patient outcomes and the management of chronic infections.

Overcoming the limitations of current biofilm diagnostic methods requires the development of faster, more sensitive techniques. Traditional methods often fail due to biofilm complexity and slow bacterial growth. Emerging technologies, such as real-time imaging, high-throughput sequencing, and biofilm-specific biomarkers, offer enhanced detection capabilities, promising more accurate and timely diagnoses for biofilm-related infections. The growing prevalence of biofilm-associated infections underscores the urgent need for rapid, sensitive, and specific diagnostic tools. Traditional diagnostic methods, including culturing and microscopy, often fail to detect biofilms due to their unique structure, slow growth, and heterogeneous nature. These limitations result in delayed diagnoses and ineffective treatments, especially in chronic infections. Therefore, developing advanced diagnostic techniques that can quickly identify biofilms, differentiate between viable and non-viable cells, and detect biofilm-associated pathogens with high specificity is essential. Emerging technologies such as real-time imaging, high-throughput sequencing, and biofilm-specific biomarkers show great promise in improving diagnostic accuracy, enabling timely interventions, and ultimately enhancing patient outcomes.

Integrating advanced diagnostic technologies for biofilm-associated infections into clinical practice is essential for effective management. Techniques such as real-time imaging, high-throughput sequencing, and biofilm-specific biomarkers can significantly improve detection accuracy and guide targeted treatments. This integration enables timely diagnoses, personalized therapeutic approaches, and better outcomes for patients with biofilm-related infections. Personalized treatment approaches for biofilm-associated infections rely on accurate diagnostic data to tailor therapies to individual patient needs. By identifying specific biofilm characteristics, such as microbial composition, resistance mechanisms, and structural features, clinicians can select targeted antimicrobial agents or alternative therapies. This approach enhances treatment efficacy, reduces the risk of recurrence, and minimizes unnecessary side effects, improving patient outcomes in chronic biofilm-related infections.

XII. Future trends in biofilm diagnostics (AI, machine learning, and data integration)

The future of biofilm diagnostics is poised to be revolutionized by the integration of artificial intelligence (AI), machine learning (ML), and advanced data analytics. Traditional diagnostic methods, such as culturing, microscopy, and PCR, have limitations in detecting and characterizing biofilms, particularly due to their complex, heterogeneous nature and slow metabolic states. However, AI and ML have the potential to overcome these challenges by enhancing the sensitivity, accuracy, and speed of biofilm detection and analysis [55].

Machine learning algorithms can be trained to identify patterns in large datasets derived from biofilm-related studies, including genomic, proteomic, and imaging data. These algorithms can facilitate the identification of biofilm-specific biomarkers and predict resistance profiles based on biofilm structure and microbial composition. AI-powered imaging analysis tools, such as deep learning techniques, can rapidly process microscopic and SEM images, enabling automated detection of biofilm formation and quantification of biofilm structures. Moreover, AI and ML can aid in the integration of various diagnostic data types, such as genomic sequencing, bioinformatics, and real-time imaging, into a unified diagnostic approach. This data fusion would provide comprehensive insights into biofilm characteristics, including microbial diversity, antibiotic resistance mechanisms, and the dynamics of biofilm formation and dispersal. By integrating these diagnostic technologies with clinical practices, clinicians will be better equipped to provide personalized, targeted therapies for biofilm-related infections. The use of AI and ML in biofilm diagnostics also holds promise for predicting infection outcomes and treatment responses, allowing for the optimization of therapeutic strategies and the reduction of unnecessary treatments. As these technologies evolve, they will enable faster, more accurate, and personalized diagnosis of biofilm-associated infections, improving patient outcomes and advancing the field of infectious disease management.

XIII. Conclusion

Bacteria within biofilms often exhibit slower growth rates and adopt distinct metabolic states, which contribute to their reduced susceptibility to antibiotics designed to target rapidly dividing cells. These challenges are further compounded by the heterogeneous microenvironments present within biofilms and the potential for genetic alterations that enhance antibiotic resistance. Biofilms are commonly found on medical implants and devices, such as catheters and prosthetic joints, where they pose significant challenges in various clinical contexts. These biofilm-associated chronic infections are notoriously difficult to treat. In addition to their role in medical device-related infections, biofilms are implicated in a range of other chronic conditions, including gastrointestinal, respiratory, urinary tract infections, and chronic otitis media. The objective of this review is to examine the complex challenges presented by biofilms in chronic infections, particularly their contribution to antibiotic resistance, and their broader impact across diverse medical settings.

Biofilm-associated infections pose significant challenges in both diagnosis and treatment due to their complex structure and inherent resistance mechanisms. Biofilms are dense microbial communities encased in an extracellular matrix that protects bacteria from host immune responses and antimicrobial agents. This makes biofilm-related infections, often associated with chronic conditions and implanted medical devices, difficult to manage and treat effectively. Traditional diagnostic methods, including culturing and microscopy, struggle to detect biofilms due to their slow-growing nature and complex three-dimensional architecture. Advanced techniques such as scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), and molecular assays like PCR provide improved detection, but they still face limitations in capturing the full complexity of biofilm infections and often require specialized equipment or lengthy processing times. The primary challenge in diagnosing biofilm-associated infections lies in accurately detecting and characterizing biofilms, given their heterogeneous nature and resistance to conventional diagnostic tools. Current methods may not identify all bacterial species within a biofilm, and the slow growth or dormant state of biofilm bacteria may lead to false-negative results. Additionally, the inability of many diagnostic approaches to distinguish biofilm from planktonic bacteria further complicates accurate detection.

Looking forward, the integration of advanced diagnostic technologies, such as real-time imaging, high-throughput sequencing, artificial intelligence, and machine learning, holds great promise in addressing these challenges. These technologies can improve the sensitivity, speed, and accuracy of biofilm detection and help clinicians better understand biofilm characteristics, including resistance profiles and microbial diversity. The development of personalized, data-driven treatment strategies will offer more targeted approaches for managing biofilm-related infections, ultimately leading to improved patient outcomes and more effective management of chronic infections. Continued innovation and research are essential to advancing biofilm diagnostics and therapeutic strategies.

XIV. References

1. Campoccia D, Mirzaei R, Montanaro L, Arciola CR (2019) Hijacking of immune defences by biofilms: a multifront strategy. Biofouling 35:1055–1074
2. Rasoul M, Rokhsareh M, Mohammad SM, Sajad K, Ahmadreza M (2019) The Human Immune System against Staphylococcus epidermidis. Crit Rev Immunol 39:151–163
3. Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA (2018) Bacterial biofilm and associated infections. Journal of the Chinese Medical Association 81:7–11
4. Mirzaei R, Mirzaei H, Alikhani MY, et al (2020) Bacterial biofilm in colorectal cancer: What is the real mechanism of action? Microb Pathog 142:104052
5. Dhadse P, Gattani D, Mishra R (2010) The link between periodontal disease and cardiovascular disease: How far we have come in last two decades ? J Indian Soc Periodontol 14:148
6. Hardie JM (1992) Oral microbiology: current concepts in the microbiology of dental caries and periodontal disease. Br Dent J 172:271–278
7. Singh S, Singh SK, Chowdhury I, Singh R (2017) Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. Open Microbiol J 11:53–62
8. Vestby LK, Grønseth T, Simm R, Nesse LL (2020) Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics 9:59
9. Boisvert A-A, Cheng MP, Sheppard DC, Nguyen D (2016) Microbial Biofilms in Pulmonary and Critical Care Diseases. Ann Am Thorac Soc 13:1615–1623
10. Zhao A, Sun J, Liu Y (2023) Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. https://doi.org/10.3389/fcimb.2023.1137947
11. H∅iby N, Ciofu O, Bjarnsholt T (2010) Pseudomonas Aeruginosa Biofilms in Cystic fibrosis. Future Microbiol 5:1663–1674
12. Khan J, Tarar SM, Gul I, Nawaz U, Arshad M (2021) Challenges of antibiotic resistance biofilms and potential combating strategies: a review. 3 Biotech 11:169
13. Zhao G, Usui ML, Lippman SI, James GA, Stewart PS, Fleckman P, Olerud JE (2013) Biofilms and Inflammation in Chronic Wounds. Adv Wound Care (New Rochelle) 2:389–399
14. Liu Y, Yang S-F, Li Y, Xu H, Qin L, Tay J-H (2004) The influence of cell and substratum surface hydrophobicities on microbial attachment. J Biotechnol 110:251–256
15. Donlan RM (2001) Biofilm Formation: A Clinically Relevant Microbiological Process. Clinical Infectious Diseases 33:1387–1392
16. Guzmán-Soto I, McTiernan C, Gonzalez-Gomez M, Ross A, Gupta K, Suuronen EJ, Mah T-F, Griffith M, Alarcon EI (2021) Mimicking biofilm formation and development: Recent progress in in vitro and in vivo biofilm models. iScience 24:102443
17. Tolker-Nielsen T (2015) Biofilm Development. Microbiol Spectr. https://doi.org/10.1128/microbiolspec.MB-0001-2014
18. Jiang Z, Nero T, Mukherjee S, Olson R, Yan J (2021) Searching for the Secret of Stickiness: How Biofilms Adhere to Surfaces. Front Microbiol. https://doi.org/10.3389/fmicb.2021.686793
19. de Sousa T, Hébraud M, Dapkevicius MLNE, Maltez L, Pereira JE, Capita R, Alonso-Calleja C, Igrejas G, Poeta P (2021) Genomic and Metabolic Characteristics of the Pathogenicity in Pseudomonas aeruginosa. Int J Mol Sci 22:12892
20. Qu D, Hou Z, Li J, et al (2020) A new coumarin compound DCH combats methicillin-resistant Staphylococcus aureus biofilm by targeting arginine repressor. Sci Adv. https://doi.org/10.1126/sciadv.aay9597
21. Landry BP, Palanki R, Dyulgyarov N, Hartsough LA, Tabor JJ (2018) Phosphatase activity tunes two-component system sensor detection threshold. Nat Commun 9:1433
22. Dixit D, Soppina V, Ghoroi C (2019) A Non-electric and Affordable Surface Engineered Particle (SEP) based Point-of-Use (POU) Water Disinfection System. Sci Rep 9:18245
23. Palmqvist N, Foster T, Fitzgerald JR, Josefsson E, Tarkowski A (2005) Fibronectin-Binding Proteins and Fibrinogen-Binding Clumping Factors Play Distinct Roles in Staphylococcal Arthritis and Systemic Inflammation. J Infect Dis 191:791–798
24. Dapunt U, Prior B, Oelkrug C, Kretzer JP (2020) IgY Targeting Bacterial Quorum-Sensing Molecules in Implant-Associated Infections. Molecules 25:4027
25. Guttenplan SB, Kearns DB (2013) Regulation of flagellar motility during biofilm formation. FEMS Microbiol Rev 37:849–871
26. Elawady R, Aboulela AG, Gaballah A, Ghazal AA, Amer AN (2024) Correction: Antimicrobial Sub-MIC induces Staphylococcus aureus biofilm formation without affecting the bacterial count. BMC Infect Dis 24:1194
27. Schaeffer CR, Hoang T-MN, Sudbeck CM, Alawi M, Tolo IE, Robinson DA, Horswill AR, Rohde H, Fey PD (2016) Versatility of Biofilm Matrix Molecules in Staphylococcus epidermidis Clinical Isolates and Importance of Polysaccharide Intercellular Adhesin Expression during High Shear Stress. mSphere. https://doi.org/10.1128/mSphere.00165-16
28. Milles LF, Unterauer EM, Nicolaus T, Gaub HE (2018) Calcium stabilizes the strongest protein fold. Nat Commun 9:4764
29. Shi T, Hou X, Guo S, Zhang L, Wei C, Peng T, Hu X (2021) Nanohole-boosted electron transport between nanomaterials and bacteria as a concept for nano–bio interactions. Nat Commun 12:493
30. Bannoehr J, Ben Zakour NL, Reglinski M, et al (2011) Genomic and Surface Proteomic Analysis of the Canine Pathogen Staphylococcus pseudintermedius Reveals Proteins That Mediate Adherence to the Extracellular Matrix. Infect Immun 79:3074–3086
31. Wang X, Liu M, Yu C, Li J, Zhou X (2023) Biofilm formation: mechanistic insights and therapeutic targets. Molecular Biomedicine 4:49
32. Azeem K, Fatima S, Ali A, Ubaid A, Husain FM, Abid M (2025) Biochemistry of Bacterial Biofilm: Insights into Antibiotic Resistance Mechanisms and Therapeutic Intervention. Life 15:49
33. Sharma D, Misba L, Khan AU (2019) Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control 8:76
34. Klausen M, Heydorn A, Ragas P, Lambertsen L, Aaes-Jørgensen A, Molin S, Tolker-Nielsen T (2003) Biofilm formation by Pseudomonas aeruginosa wild type, flagella and type IV pili mutants. Mol Microbiol 48:1511–1524
35. Silva NBS, Marques LA, Röder DDB (2021) Diagnosis of biofilm infections: current methods used, challenges and perspectives for the future. J Appl Microbiol 131:2148–2160
36. Høiby N, Bjarnsholt T, Moser C, et al (2015) ESCMID∗ guideline for the diagnosis and treatment of biofilm infections 2014. Clinical Microbiology and Infection 21:S1–S25
37. Dhiman S, Kumar A, Kaur G, Mukherjee G, Rustagi S, Shreaz S, Negi R, Yadav AN (2024) Bacterial biofilms: Pathogenesis, monitoring, treatment approaches and associated challenges. Biologia (Bratisl) 79:3161–3181
38. Wu J, Xi C (2009) Evaluation of Different Methods for Extracting Extracellular DNA from the Biofilm Matrix. Appl Environ Microbiol 75:5390–5395
39. Serrage HJ, Jepson MA, Rostami N, Jakubovics NS, Nobbs AH (2021) Understanding the Matrix: The Role of Extracellular DNA in Oral Biofilms. Frontiers in Oral Health. https://doi.org/10.3389/froh.2021.640129
40. Kaur S, Bran L, Rudakov G, Wang J, Verma MS (2025) Propidium Monoazide is Unreliable for Quantitative Live–Dead Molecular Assays. Anal Chem 97:2914–2921
41. Golpayegani A, Douraghi M, Rezaei F, Alimohammadi M, Nodehi RN (2019) Propidium monoazide–quantitative polymerase chain reaction (PMA-qPCR) assay for rapid detection of viable and viable but non-culturable (VBNC) Pseudomonas aeruginosa in swimming pools. J Environ Health Sci Eng 17:407–416
42. Carini P, Marsden PJ, Leff JW, Morgan EE, Strickland MS, Fierer N (2016) Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat Microbiol 2:16242
43. Browne DJ, Kelly AM, Brady JL, Doolan DL (2022) A high-throughput screening RT-qPCR assay for quantifying surrogate markers of immunity from PBMCs. Front Immunol. https://doi.org/10.3389/fimmu.2022.962220
44. Pérez-Cobas AE, Gomez-Valero L, Buchrieser C (2020) Metagenomic approaches in microbial ecology: an update on whole-genome and marker gene sequencing analyses. Microb Genom. https://doi.org/10.1099/mgen.0.000409
45. Lema NK, Gemeda MT, Woldesemayat AA (2023) Recent Advances in Metagenomic Approaches, Applications, and Challenges. Curr Microbiol 80:347
46. Mir DH, Rather MA (2024) Advantages and Limitations of the Biofilm Study Methods. Appl Biochem Microbiol 60:264–279
47. Singhal N, Kumar M, Kanaujia PK, Virdi JS (2015) MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00791

48. Mlynáriková K, Šedo O, Růžička F, Zdráhal Z, Holá V, Mahelová M (2016) Evaluation of capacity to detect ability to form biofilm in Candida parapsilosis sensu stricto strains by MALDI-TOF MS. Folia Microbiol (Praha) 61:465–471
49. Silva NBS, Marques LA, Röder DDB (2021) Diagnosis of biofilm infections: current methods used, challenges and perspectives for the future. J Appl Microbiol 131:2148–2160
50. Zhou L, Zhang Y, Ge Y, Zhu X, Pan J (2020) Regulatory Mechanisms and Promising Applications of Quorum Sensing-Inhibiting Agents in Control of Bacterial Biofilm Formation. Front Microbiol. https://doi.org/10.3389/fmicb.2020.589640
51. Di Bonaventura G, Pompilio A (2021) In Vitro Antimicrobial Susceptibility Testing of Biofilm-Growing Bacteria: Current and Emerging Methods. pp 33–51
52. Arzmi MH, Kamil WNWA, Zainal M (2023) Monospecies and polymicrobial biofilms in static and flow environment. pp 51–66
53. Olsen I (2015) Biofilm-specific antibiotic tolerance and resistance. European Journal of Clinical Microbiology & Infectious Diseases 34:877–886
54. Verma P (2017) Minimum Biofilm Eradication Concentration (MBEC) Assay of Silver and Selenium Nanoparticles against Biofilm forming Staphylococcus aureus. Journal of Medical Science And clinical Research. https://doi.org/10.18535/jmscr/v5i4.77
55. Abeyrathna D, Ashaduzzaman M, Malshe M, Kalimuthu J, Gadhamshetty V, Chundi P, Subramaniam M (2022) An AI-based approach for detecting cells and microbial byproducts in low volume scanning electron microscope images of biofilms. Front Microbiol. https://doi.org/10.3389/fmicb.2022.996400