STRUCTURE, INFECTION DYNAMICS, AND CONTROL STRATEGIES OF BACTERIAL BIOFILMS

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PREFACE

Bacterial biofilms have emerged as one of the most critical research domains in modern microbiology, gaining increasing significance in both fundamental science and clinical practice. These structures, which arise when microorganisms adhere to surfaces and organize into multilayered communities embedded within a protective extracellular matrix, lie at the center of contemporary health challenges such as antibiotic resistance, the persistence of chronic infections, and therapeutic failure. In particular, the biofilm-forming capacity of ESKAPE pathogens substantially exacerbates the difficulties encountered in managing infectious diseases and necessitates the pursuit of novel intervention strategies.

This book provides a comprehensive examination of the structural and functional characteristics of biofilms, their developmental stages, quorum-sensing–based communication mechanisms, and the biological outcomes of these processes that contribute to antimicrobial resistance. Furthermore, it delineates the limitations of conventional control approaches and critically evaluates current literature on next-generation therapeutic strategies targeting the biofilm matrix. Spanning a broad spectrum from natural compounds and nanotechnological applications to matrix-degrading enzymes and quorum-sensing inhibitors these approaches offer innovative solutions aimed at disrupting biofilm integrity and restoring bacterial susceptibility.

In this context, I believe that the book will offer substantial contributions to researchers, clinicians, and all scholars interested in understanding, analyzing, and developing effective strategies against biofilm-associated infections. Considering the pivotal role of biofilms in microbial ecology, antimicrobial resistance, and clinical infectious diseases, it is evident that any academic work in this field holds critical value for the future of infection control and therapeutic success.

24/11/2025

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INTRODUCTION

The biofilm provides a protective environment for bacterial cells against antibiotic activity, host immune responses, nutrient limitations, and various environmental stresses (Rather et al., 2021). Composed of components such as carbohydrates, lipids, proteins, and extracellular nucleic acids (eDNA), this structure primarily consists of two main elements: extracellular polymeric substances (EPS) and bacterial cell communities (J. Li et al., 2019; Yi et al., 2019). Biofilm formation is considered one of the fundamental strategies employed by bacteria to survive under adverse conditions and adapt to the host (Koo et al., 2017).

Biofilm development generally occurs as a five-stage cyclical process: initial surface attachment (reversible followed by irreversible adhesion), EPS synthesis, biofilm maturation, and the dispersion of cells to colonize new surfaces (Rather et al., 2021; Sauer et al., 2022). After adhering to biotic or abiotic surfaces, bacteria secrete EPS, encapsulating themselves within a protective matrix. As the cell population increases, the matrix thickens, leading to the development of a mature biofilm. Cells that disperse from the mature biofilm attach to new surfaces, thereby initiating the cycle anew (Sauer et al., 2022).

This process is regulated by the quorum sensing (QS) mechanism, which controls communication among bacterial cells (Kameswaran et al., 2024). QS enables bacteria to coordinate gene expression and metabolic activities in response to population density. Through this mechanism, the production of EPS-composed of lipids, polysaccharides, proteins, eDNA, and ions-occurs in a synchronized manner at the community level (Yi et al., 2019). This physio-metabolic shift confers resistance to desiccation, antimicrobial agents, and host immune responses in bacteria (Preda et al., 2019).

Biofilms often comprise multiple bacterial species, resulting in polymicrobial communities (Anju et al., 2022; Fang et al., 2020; Wicaksono et al., 2022). Close cell-to-cell contact and the EPS matrix facilitate horizontal gene transfer, providing a conducive environment for the rapid dissemination of antibiotic resistance genes (Michaelis et al., 2023). Therefore, biofilms are considered a significant reservoir for multidrug-resistant (MDR) bacteria (Khan et al., 2021). Infections associated with MDR bacteria are difficult to treat, often chronic, and frequently result in fatal clinical outcomes. The Centers for Disease Control and Prevention (CDC) report that over 2 million infections and approximately 23,000 deaths occur annually due to MDR bacteria (CDC, 2019).

ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) are particularly associated with biofilm formation (De Oliveira et al., 2020). Infections caused by these pathogens are typically chronic and exhibit resistance to treatment (Schulze et al., 2021). Biofilm-associated infections are commonly observed in the lungs of patients with cystic fibrosis, surgical wounds, orthopedic implants, and on intravenous and urinary catheters (Su et al., 2022). The biofilm structure hinders antibiotic penetration, enhances efflux pump activity, induces target modifications, and contributes to the formation of persistent cells (Halawa et al., 2023; Upadhyay et al., 2025).

Biofilms play a role not only in the development of antibiotic resistance but also in the persistence of chronic infections and, potentially, in the progression of certain cancer types. Some studies indicate that biofilms can release biological molecules such as polyamines, influencing toxin production and carnitine metabolism, processes that may be associated with cellular proliferation and carcinogenesis (Upadhyay et al., 2025).

Consequently, therapeutic strategies targeting biofilms constitute a central focus of current antimicrobial research. Since the eradication of biofilm-associated infections is highly challenging, studies aim to target the early stages of biofilm development (Delik et al., 2023). Within this context, modulation of the QS mechanism is considered a promising approach (Y. Li et al., 2023). In cases where preventive strategies prove insufficient, the EPS matrix is targeted to enhance the susceptibility of pathogenic strains to antibiotics (Mirghani et al., 2022; Ramakrishnan et al., 2022). Biotechnology and nanotechnology-based approaches have attracted significant attention due to their potential to enhance the efficacy of conventional antibiotics and to restore susceptibility in resistant strains (Liu et al., 2022; Sheridan et al., 2022).

In conclusion, biofilm formation represents a complex defense mechanism developed by bacteria to withstand environmental stress conditions. Given its central role in antimicrobial resistance, chronic infections, and therapeutic failure, the development of novel treatment strategies targeting biofilms is of paramount importance for future infection control approaches.

1. Structure and Functional Dynamics of the Bacterial Biofilm Matrix

Microorganisms organize into biofilm communities within a three-dimensional EPS matrix that they synthesize themselves and that surrounds the cells. This matrix provides the structural integrity, functional flexibility, and environmental adaptability of the biofilm (Flemming et al., 2024).

The primary components of the biofilm matrix include polysaccharides, proteins, eDNA, lipids, and lipoproteins. The main components and structural roles of the biofilm matrix are summarized in Table 1. Polysaccharides, as the major constituents of EPS, facilitate intercellular adhesion and surface attachment. The three-dimensional structure of the matrix is supported by structural proteins and amyloid like fibers. eDNA contributes to the matrix’s volume and aids in maintaining its structural stability (Campoccia et al., 2021) The hydrophobic properties and barrier function of the matrix are supported by membrane vesicles and lipids (Flemming et al., 2022).

Table 1. Major Components of the Biofilm Matrix and Their Structural Roles.

Matrix Component	Structural Function and Characteristics	Key Features	References
Polysaccharides	Main scaffold, viscoelastic properties, cohesion, and layering	Surface attachment, protection	(Saharan et al., 2024)
Proteins	Filamentous fibers, cross-links, and amyloid structures	Mechanical strength, scaffold	(Kavanaugh et al., 2019)
eDNA	Structural stability, interactions with proteins and polysaccharides	Matrix integrity, gene transfer	(Secchi et al., 2022)

Lipids / Lipoproteins	Hydrophobic barrier, cross-linking with eDNA	Barrier function, stability	(Böhning et al., 2024)
Water	Constitutes ~90% of the matrix, nutrient transport and diffusion	Metabolic activity, diffusion	(Saharan et al., 2024)

Physically, the biofilm matrix exhibits both viscous and elastic behavior. The mechanical resistance of the biofilm to stress and its capacity for deformation are determined by the polymeric nature of EPS. Biofilm adhesion, stiffness, and cohesion are directly influenced by the quantity and composition of EPS (Hasan et al., 2024). Moreover, the layered structure of the matrix allows for the spatial segregation of different EPS components and microbial communities within the biofilm (Moreau et al., 2025; Xin et al., 2025).

Microorganisms are protected in multiple ways by the biofilm matrix. EPS shields the cells by forming a physical barrier against antimicrobial agents and the immune system (Karygianni et al., 2020). The matrix facilitates the retention and storage of nutrients, thereby enhancing the resilience of cells within the biofilm to environmental changes (Yin et al., 2019). Additionally, extracellular enzymes within the matrix function similarly to an external digestive system, breaking down various nutrients and making them available to the cells (Flemming et al., 2022).

The matrix facilitates intercellular communication, including quorum sensing and signaling molecules, while also coordinating gene expression (Wong et al., 2022). The structural properties of the matrix confer resistance to the biofilm against environmental stresses such as pH, temperature, and toxic substances (Flemming et al., 2024).

Environmental stress and changes lead to the continuous remodeling of the biofilm matrix. The physical properties and components of the matrix can be influenced by antibiotic stress, nutrient limitation, and other environmental factors. For example, as a result of phosphorus or nitrogen limitation, the polysaccharide and eDNA content of EPS may increase, leading to a denser and more homogeneous matrix structure (Desmond et al., 2017). The remodeling and modification of the matrix under stress support the survival and adaptation of the biofilm (Moreau et al., 2025).

As the biofilm matures or encounters environmental signals, matrix components such as polysaccharides and proteins are enzymatically degraded. This process leads to the detachment of cells from the biofilm, initiating the dispersal phase (Pandit et al., 2020). These dynamic processes determine both the stability of the biofilm and its ability to respond to environmental opportunities (Wong et al., 2022).

2. Mechanisms of Bacterial Biofilm Formation and the Role of Quorum Sensing

Biofilm development is a multi-stage process. In the initial stage, bacteria attach reversibly to substrates such as dental surfaces or medical implants, remaining vulnerable to antibiotics during this period. Subsequently, bacteria produce EPS, adhere irreversibly to the surface, proliferate, and form colonies. During the maturation phase, the biofilm acquires a mushroom-like three-dimensional structure that can reach up to 50 µm in thickness (Alexander et al., 2016). During this process, factors such as twitching motility, cell signaling, and environmental conditions shape the architecture of the biofilm (Stoodley et al., 2002). Mature biofilms possess water channels that facilitate nutrient and metabolite transport and exhibit an organization reminiscent of primitive multicellular organisms. In the final stage, portions of the biofilm dissolve, allowing bacteria to become free and establish colonies on new surfaces (E. A. George et al., 2007).

The QS mechanism plays a critical role in regulating biofilm formation. QS is a cell-to-cell communication system based on chemical signaling that enables bacteria to regulate gene expression in response to population density (Omwenga et al., 2023). This system operates through the synthesis and detection of small signaling molecules called autoinducers (AIs). Through the QS mechanism, bacteria coordinate behaviors such as virulence factor expression, toxin production, and biofilm development (Zhou et al., 2020).

In Gram-negative bacteria, QS is typically mediated by N-acyl homoserine lactone (AHL) molecules. *P. aeruginosa* activates the LasR and RhlR receptors through signaling molecules (OdDHL and BHL) synthesized by the lasI and rhlI genes, thereby regulating biofilm formation and the expression of virulence genes (Dekimpe et al., 2009). The Las system controls the production of factors such as elastase, alkaline protease, and exotoxin A, which enhance the structural integrity of the biofilm and the pathogenicity of the bacterium. The Rhl system regulates swarming motility and pyocyanin production, thereby promoting colonization and increasing the potential for damage to host tissue (Omwenga et al., 2023).

Although *Escherichia coli* lacks the gene for AHL synthesis, it possesses a receptor called SdiA that can detect AHLs produced by other species. Through this receptor, *E. coli* regulates biofilm-associated processes such as EPS production and surface attachment (Jamuna Bai et al., 2016). Additionally, many Gram-negative bacteria engage in interspecies communication using AI-2 or AI-3 systems. In *V. cholerae*, the AI-2 signal is detected via the LuxPQ receptor complex, and high levels of AI-2 suppress biofilm progression (Anderson et al., 2015).

In Gram-positive bacteria, the signaling molecules are typically autoinducing peptides (AIPs). In *S. aureus*, the agr system (comprising the agrA, agrB, agrC, agrD, and hld genes) regulates the maturation and dispersal stages of the biofilm. agr mutants form thicker and more resilient biofilms due to a reduced ability to detach from mature biofilms (Eric Omori Omwenga et al., 2024).

These mechanisms clearly demonstrate the central regulatory role of bacterial QS in biofilm formation. A detailed understanding of QS systems is crucial for the development of novel therapeutic strategies targeting these communication networks. In particular, QS antagonists have the potential to inhibit biofilm formation by blocking signal transduction and represent promising alternatives in the treatment of antibiotic-resistant infections (Jiang et al., 2019).

3. Modulation of Host Immune Response and Evasion Mechanisms in Biofilm Formation

Bacterial biofilms, a primary cause of chronic infections, protect themselves from the host immune system and antibiotics by modulating immune responses and employing various evasion mechanisms. Biofilm-associated bacteria exhibit phenotypes distinct from planktonic bacteria, reducing the effectiveness of the immune response (Peng et al., 2022; Sahu et al., 2025).

Biofilms can modulate the host immune system at both adaptive and innate levels. In innate immunity, biofilms reduce the activity of neutrophils and macrophages. A study reported that *S. aureus* biofilms promote bacterial persistence by directing macrophages toward an anti-inflammatory and pro-fibrotic M2 phenotype (Mirzaei et al., 2022). Biofilms hinder the access of immune cells to these structures by impairing neutrophil chemotaxis and inhibiting the formation of neutrophil extracellular traps (Batoni et al., 2021; Cangui-Panchi et al., 2023).

In adaptive immunity, biofilm infections generally become chronic by disrupting the Th1/Th2 balance and rendering the antibody response ineffective. In particular, *P. aeruginosa* biofilms cause tissue damage and prevent the clearance of infection (Thomsen et al., 2022). Additionally, biofilms enhance the production of immunosuppressive cytokines such as IL-10, which attenuates the inflammatory response (Cruickshank et al., 2024; Van Roy et al., 2025).

Biofilms employ multiple mechanisms to evade host defenses, including the extracellular matrix barrier, protease and toxin secretion, cytokine modulation, phenotypic heterogeneity and persister cells, as well as the regulation of virulence factors. The extracellular matrix barrier prevents the penetration of antimicrobial agents and immune cells into the biofilm (Mathew et al., 2023). The secretion of bacterial proteases and toxins degrades immunoglobulins and components of the complement system, thereby weakening the immune response (Ramírez-Larrota et al., 2022). The induction of anti-inflammatory cytokines such as IL-10 reduces the microbicidal activity of macrophages and other immune cells (Van Roy et al., 2025). Bacterial biofilms evade both antibiotics and the immune system by exhibiting phenotypic heterogeneity and forming dormant persister cells (Peng et al., 2023). In particular, species such as *S. aureus* and *S. epidermidis* evade phagocytosis and complement activation through surface proteins and polysaccharides (Le et al., 2018).

Biofilm infections contribute to the persistence of chronic inflammation, which in turn impairs tissue repair. In conditions such as chronic wounds and cystic fibrosis, biofilms can adversely affect both tissue integrity and the immune response (Thomsen et al., 2022).

A comprehensive understanding of how biofilm formation exploits immune evasion mechanisms is crucial for the development of novel therapeutic strategies. Emerging approaches that target the biofilm matrix show promise in the treatment of chronic biofilm-associated infections (Ge et al., 2024; Sahu et al., 2025).

4. Clinical Significance of Biofilms and Medical Device Associated Biofilms

Biofilms are microbial communities that exhibit high resistance to antimicrobial treatments and host immune responses. These structures play a crucial role in the pathogenesis of chronic infections characterized by prolonged inflammation, such as chronic wound infections and osteomyelitis, which show tendencies for treatment resistance and recurrence (Diban et al., 2023; Masters et al., 2019).

In modern healthcare, the majority of hospital-acquired infections originate from biofilms associated with medical devices. It has been reported that 60–80% of nosocomial infections arise from biofilms developing on devices such as catheters, prosthetic joint materials, heart valves, orthopedic implants, endoscopes, and stents (Mishra et al., 2024; S. Sharma et al., 2023). Similarly, it has been reported that approximately 65% of medical device–associated infections originate from biofilms (Khatoon et al., 2018). These biofilms can lead to serious clinical syndromes such as prosthetic joint infections, endovascular infections, CLABSI, and CAUTI, often necessitating the removal of the device (Bouhrour et al., 2024; Caldara et al., 2022). A study reported biofilm colonization on central venous catheters ranging up to 81% within 1–14 days (Bouhrour et al., 2024).

Biofilm formation begins when bacteria adhere to a “conditioning film” formed by the accumulation of proteins and cellular materials on the device surface (P. Li et al., 2023; Mishra et al., 2024). Subsequently, the synthesis of the EPS matrix leads to the development of a mature biofilm structure. Bacteria within this structure exhibit 100- to 1000-fold greater antibiotic tolerance compared to their planktonic counterparts (Di Domenico et al., 2022). The primary mechanisms underlying this resistance include the inhibition of antibiotic penetration by the EPS matrix, low metabolic activity, enzymatic inactivation, and the presence of persister cells (Bouhrour et al., 2024). The reactivation of persister cells after treatment leads to the recurrence and spread of infections (D. Sharma et al., 2019). The EPS structure also diminishes the effectiveness of phagocytic cells and triggers a chronic inflammatory response, leading to tissue damage (Ramírez-Larrota et al., 2022).

The diagnosis of biofilm-associated infections can be challenging, as many bacteria within biofilms exist in a “viable but non-culturable” state (Percival et al., 2015). Clinical manifestations are often non-specific and resemble those of other infections (Mendhe et al., 2023). Therefore, molecular methods, ultrasonography, MRI, biosensor-based approaches, and advanced imaging techniques are increasingly important for the detection of biofilms (Amod et al., 2025; Sahoo et al., 2024).

During treatment, conventional antibiotic therapies often fail, and in many cases, the infected device must be completely removed (Khatoon et al., 2018). Local applications, such as catheter lock solutions, and prolonged high-dose antibiotic treatments achieve only partial success (Wi et al., 2018). Therefore, strategies involving surface modifications with non-antibiotic agents, antifouling and antimicrobial coatings, enzymes, nanoparticles, quorum-sensing inhibitors, and bacteriophages have been intensively investigated in recent years (Mishra et al., 2024). The biocompatibility and long-term efficacy of these novel approaches need to be evaluated for clinical application (Scalia et al., 2025).

5. In Vitro Methods Used for the Evaluation of Biofilm Formation

Studying bacterial biofilm formation is crucial in both clinical and research settings to guide infection management and to develop novel anti-biofilm strategies. Techniques used for the detection and characterization of biofilms allow the examination of their structural, functional, and viability properties from various perspectives. Currently, methods for biofilm assessment include a wide range of approaches, such as measuring biomass, evaluating metabolic activity, determining viable cell counts, and performing structural and chemical analyses (Funari et al., 2022; Haney et al., 2018). While each method in biofilm research has its advantages and limitations, a combination of multiple approaches is generally preferred (Cleaver et al., 2023).

5.1. Congo Red Agar Method

The Congo Red Agar (CRA) method is a widely used, cost-effective, and practical screening technique for the phenotypic detection of bacterial biofilm and slime layer production. This method enables the rapid assessment of the biofilm-forming capacity of many clinical isolates, particularly species of *Staphylococcus* (Anan et al., 2024; Harika et al., 2020).

CRA is a specialized medium composed of brain heart infusion agar, sucrose, and Congo red dye. After bacterial isolates are inoculated onto this medium, they are incubated at 37°C for 24–48 hours (Figure 1). Biofilm-producing bacteria synthesize polysaccharides that react with Congo red, forming black, dry-crystalline colonies on the agar. Non-biofilm producers, in contrast, appear as red or pink colonies (Basnet et al., 2023).

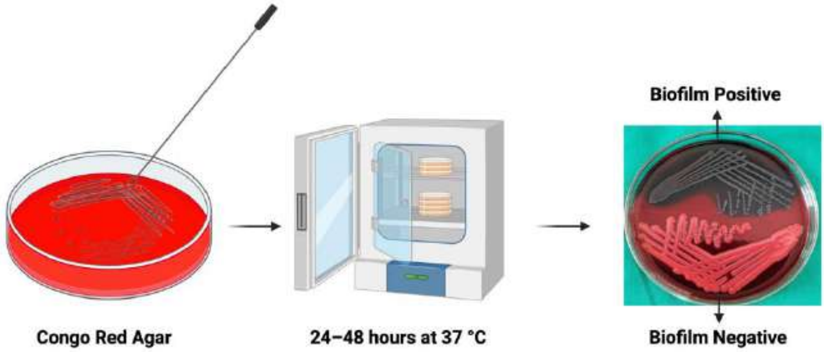

Figure 1. Appearance of biofilm-negative and biofilm-positive bacterial isolates incubated on Congo Red Agar at 37°C for 24–48 hours.

The advantages of the CRA method include its rapidity, low cost, and ease of application. It directly indicates the presence of biofilm through colony morphology and allows for the rapid screening of a large number of samples (Anan et al., 2024).

Limitations of the method include its qualitative nature, as results rely on observation and color changes, which can introduce variability in subjective assessments. Additionally, some studies have shown that CRA may yield false-negative results, particularly for weak biofilm-producing bacteria. Quantitative methods, such as the microtiter plate assay, have been reported to be more reliable than CRA (Er, 2024; Kord et al., 2018).

5.2. Standard Glass Tube Method

The standard glass tube test is one of the most commonly used, practical, and cost-effective methods for the phenotypic assessment of biofilm formation. This method allows for the rapid and visual evaluation of the biofilm-forming capacity of bacterial isolates, particularly in clinical laboratories and research settings (Furtuna et al., 2018; Gangashettappa et al., 2019; Halim et al., 2018).

The glass tube method involves inoculating a bacterial suspension into sterile glass tubes containing growth medium, followed by incubation for 24 to 48 hours. After incubation, the tube contents are discarded, and the tubes are washed several times with phosphate-buffered saline (PBS). The biofilm adhering to the inner surface of the tube is then stained with 0.1% crystal violet. Excess dye is removed by washing with PBS, and the tube is allowed to dry. The presence of a visible purple film on the inner surface indicates biofilm formation. Depending on the thickness and density of the biofilm, its amount can be classified as negative, weak, moderate, or strong (Figure 2) (Gangashettappa et al., 2019).

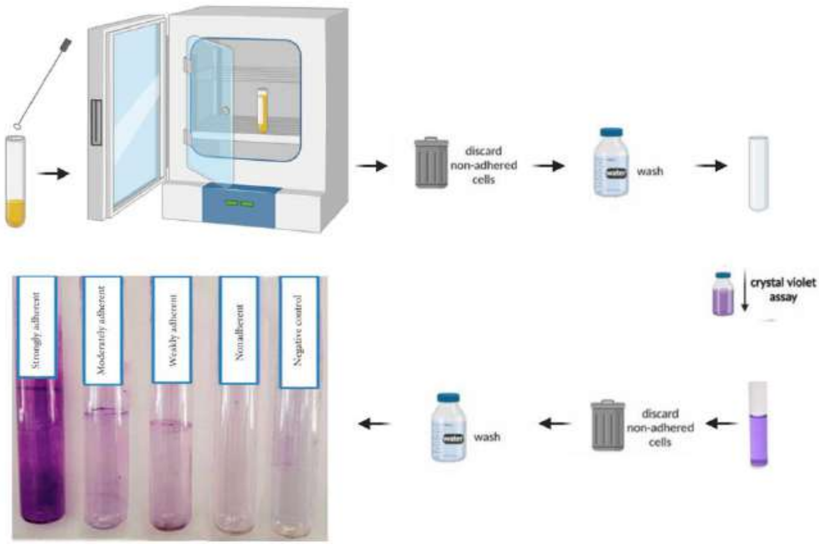

Figure 2. Procedural Steps of the Glass Tube Method for the Detection of Biofilm Formation.

The advantages of the glass tube method include its low cost, ease of use, rapidity, and the lack of need for specialized equipment. It also allows for the screening of a large number of samples in a short time (Basnet et al., 2023). Its limitations include the subjective nature of the results, which can vary depending on the observer. The method may produce false-negative results for weak biofilm-producing bacteria, and the outcomes are not quantitative (Kord et al., 2018).

Compared to the microtiter plate method, the glass tube method has been found to be less sensitive and specific in studies; however, due to its practicality, it is frequently used as a screening test. It provides reliable results, particularly for strong biofilm-producing bacteria (Basnet et al., 2023).

5.3. Methods Using Microtiter Plates

One of the most common and reliable methods for the sensitive, specific, and quantitative assessment of biofilm formation is the spectrophotometric microtiter plate (96-well microplate) assay. This method is frequently used in biofilm research, often with various modifications (Allkja et al., 2021; Thibeaux et al., 2020).

The bacterial suspension is typically incubated in 96-well microplates with an appropriate growth medium containing 1–3% glucose (De Jesus et al., 2019). After incubation, the contents of the microplate wells are removed, and the wells are washed with PBS. Sodium acetate or methanol can be used to fix the biofilm (Shukla et al., 2017). The biofilm is then stained using dyes such as crystal violet, safranin, or trypan blue (Centorame et al., 2020). After staining, the microplates are dried, and the dye is subsequently solubilized using acetic acid or acetone (T. George et al., 2025).

The optical density (OD) of each well is typically measured at 570 nm using a microplate reader. Biofilm presence and its degree are determined by comparing the OD values to those of control wells (Thibeaux et al., 2020).

The method provides quantitative results, high sensitivity, reproducibility, and the capacity for the analysis of multiple samples simultaneously (Allkja et al., 2021). During the washing steps, care must be taken not to damage the biofilm, and precautions should be taken to prevent errors such as evaporation and the "edge effect" in the outer wells (Centorame et al., 2020). The dyes used and the measurement wavelengths should be standardized (T. George et al., 2025).

5.3.1. Crystal Violet

Crystal violet staining is the most commonly used and standard method for the quantitative assessment of biofilm formation in 96-well microplates. This technique allows for the rapid, cost-effective, and efficient measurement of biofilm biomass (Andersen et al., 2024).

Bacteria should be incubated in microplate wells with an appropriate growth medium. After incubation, the wells are washed, and a 0.1–0.5% crystal violet solution is added, followed by incubation for 15–30 minutes (Altuwaijri et al., 2025; Kamimura et al., 2022). To remove excess dye, the wells are washed several times (Stiefel et al., 2016). The crystal violet bound to the biofilm is solubilized with 33% acetic acid or 94–100% ethanol, and the OD is typically measured spectrophotometrically at 570–595 nm (Figure 3) (Altuwaijri et al., 2025; T. George et al., 2025).

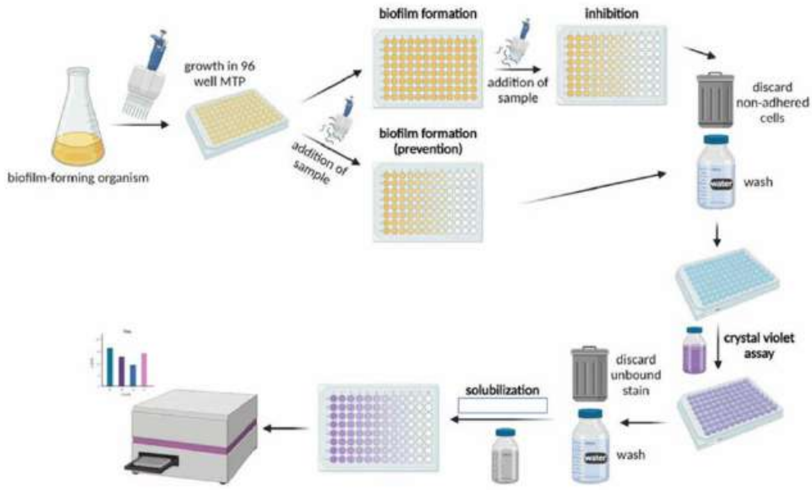

Figure 3. Procedural Steps of the Crystal Violet Method for the Detection of Biofilm Formation.

Its advantages include simplicity, low cost, reproducibility, and suitability for the simultaneous analysis of a large number of samples (Shukla et al., 2017; Thibeaux et al., 2020).

Its limitations include potential toxicity, the inability to distinguish between live and dead cells or matrix components, the "edge effect" in outer wells, and variability during washing steps (Amador et al., 2021; Kragh et al., 2019).

5.3.2. Safranin Staining

Safranin staining is a non-toxic and reliable alternative to crystal violet for the quantitative measurement of biofilm biomass. In recent years, it has gained prominence, particularly for laboratory safety and reproducibility. Safranin binds to the negatively charged components of bacterial cells and the extracellular matrix within the biofilm, staining the total biomass (Ommen et al., 2017).

A 0.5% safranin solution is typically used, and excess dye is washed away after staining. The optical density is measured spectrophotometrically at approximately 535 nm (Upadhyay et al., 2024).

Compared to crystal violet, safranin is much less toxic, offering advantages in terms of laboratory safety. Measurements performed with safranin yield results similar to those of crystal violet while providing higher reproducibility and sensitivity (Ommen et al., 2017). It has been successfully used for the analysis of bacterial and yeast biofilms across different species (Upadhyay et al., 2024). However, safranin does not differentiate between live and dead cells and measures the total biomass (Stiefel et al., 2016).

5.3.3. Use of XTT Assay

The XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay is a widely used, rapid, and reliable colorimetric method for measuring the metabolic activity of biofilm-forming bacteria and fungi. It is particularly preferred for assessing the activity of viable cells and testing antimicrobial efficacy. XTT is reduced by dehydrogenase enzymes in live cells to form a water-soluble orange formazan dye, with the color change being proportional to the cells' metabolic activity (Corte et al., 2019; Magaña-Montiel et al., 2024).

In this method, 96-well microplates are most commonly used. An electron carrier such as menadione or phenazine methosulfate is added along with the XTT solution (0.25–1 mg/mL), followed by incubation for 30 minutes to 4 hours. Absorbance is measured spectrophotometrically at 470–492 nm (Chavez-Dozal et al., 2016).

Its advantages include rapidity, high efficiency, reproducibility, and the selective measurement of live/metabolically active cells. When used alongside biomass-measuring methods such as crystal violet, it allows differentiation between biofilm viability and total biomass (Dogan et al., 2021; Ramage, 2016).

A limitation is that it measures only metabolically active cells, which may lead to underestimated results in the deeper layers of the biofilm due to low activity. Metabolic differences between species and strains can also affect the results (Dogan et al., 2021).

The addition of metabolic substrates, such as glucose or D-glutamine, can enhance sensitivity, particularly in mature biofilms (Gobor et al., 2011). Viability assessment of bacterial and fungal biofilms can be employed in antimicrobial susceptibility testing, environmental toxicity analyses, and bioplastic degradation studies (Corte et al., 2019; Magaña-Montiel et al., 2024).

5.3.4. Use of MTT

The MTT assay is a widely used colorimetric method for assessing cell viability, proliferation, and cytotoxicity in cell cultures. It provides a rapid and sensitive measurement of cellular metabolic activity, particularly in drug screening, toxicity analyses, and biofilm studies. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced by mitochondrial enzymes in viable cells to form insoluble purple formazan crystals. The resulting formazan is solubilized using a solvent such as DMSO, and absorbance is typically measured at 540–570 nm. The intensity of the color produced is proportional to the number of viable/metabolically active cells (Bahuguna et al., 2017).

In this method, 96-well plates are used, and MTT is added to the wells followed by incubation for 2–6 hours. The resulting formazan crystals are then dissolved using a solvent and measured spectrophotometrically (P. Kumar et al., 2018).

It measures only metabolically active cells; certain drugs or compounds can directly affect MTT reduction, potentially leading to inaccurate results. In agents that impair mitochondrial function, a distinction between viability and metabolic activity may not be possible (Hoogstraten et al., 2022; Malinowski et al., 2022).

Parameters such as MTT concentration, cell type, incubation time, and choice of solvent should be optimized. To ensure the reliability of results, the MTT assay is generally supported by additional viability tests (Ghasemi et al., 2023; Stindlova et al., 2025).

6. Conventional Approaches to Combat Biofilm Formation

Conventional methods, such as mechanical treatments, surface modifications, and chemical approaches, are employed to prevent biofilm formation or to eliminate existing biofilms (Schilcher et al., 2020). These strategies aim to disrupt the biofilm structure, eliminate embedded microorganisms, and prevent surface adhesion. Mechanical treatments, such as brushing and scrubbing, physically remove biofilms, thereby reducing microbial load. Surface modifications using hydrophilic polymers and antimicrobial coatings also inhibit biofilm development (String et al., 2020). Chemical agents, such as detergents, facilitate the removal of bacteria by disrupting biofilm cells (Fagerlund et al., 2020). Additionally, antimicrobial agents suppress biofilm growth, while biosurfactants disrupt existing biofilms, enhancing their susceptibility to other agents (Allegrone et al., 2021). Careful monitoring is required during the treatment process to prevent the release of pollutants and to minimize environmental impacts (Muhammad et al., 2020).

However, conventional approaches are often time-consuming, costly, and require specialized equipment. In some cases, they may be ineffective, and their applicability in sensitive environments is limited (Bayramov et al., 2017). Moreover, they lack the real-time data feedback and high accuracy offered by modern techniques and are often inadequate for the removal of complex biofilm communities (Darvishi et al., 2022).

7. Novel Approaches for the Prevention and Treatment of Biofilm Infections

In combating biofilm-associated infections, novel strategies are being developed, including the use of natural compounds, nanotechnology-based approaches, quorum sensing inhibition, enzymatic degradation, and antimicrobial photodynamic/sonodynamic therapies (Pourhajibagher et al., 2022). These methods disrupt the biofilm structure, reduce bacterial populations, suppress virulence factors, and enhance the efficacy of antibiotics (Bai et al., 2022). Additionally, by exerting targeted effects on specific bacterial species, they help limit the spread and complications of biofilm-associated infections (Hemmati et al., 2021).

7.1. Natural Compounds

Plant extracts, essential oils, and marine-derived compounds have emerged as promising natural agents for inhibiting biofilm formation. These compounds offer an alternative to synthetic drugs due to their low risk of side effects, environmentally friendly nature, and cost-effectiveness. Moreover, they hold significant potential in anti-biofilm strategies because of their efficacy against resistant strains and lower susceptibility to mutations (Nuță et al., 2021).

Extracts from neem (Azadirachta indica), eucalyptus (Eucalyptus globulus), oregano (Origanum vulgare), garlic (Allium sativum), and grape (Vitis vinifera) exhibit antimicrobial activity by disrupting bacterial cell walls and metabolism (Hochma et al., 2021). In particular, the essential oils of O. vulgare and A. sativum exhibit anti-biofilm activity by inhibiting growth and reducing inflammation in pathogens such as *E. coli, S. aureus*, and *S. enterica* (Peng et al., 2023).

Natural compounds such as quercetin, thymol, polyphenols, and curcumin also inhibit biofilm formation through their antibacterial, antioxidant, and anti-inflammatory properties (Veiko et al., 2023). Terpenoids found in plants inhibit bacterial growth and biofilm formation, and are considered potential sources for the development of new antibiotics (Kostoglou et al., 2020).

7.2. Advanced Nanotechnology-Based Strategies

Nanotechnology offers an innovative approach for the prevention and treatment of biofilm-associated infections (Sabzi et al., 2024). Nanomaterials, due to their unique physical and chemical properties, can disrupt biofilm structures and prevent pathogen adhesion to surfaces. Additionally, targeted drug delivery systems have been developed to transport antibiotics directly to biofilms, thereby reducing systemic toxicity (L. Kumar et al., 2023).

Coating medical device surfaces with nanoparticles prevents bacterial colonization and reduces the risk of infection (Varma et al., 2023). Silver, gold, zinc, copper, and iron nanoparticles exhibit potent anti-biofilm activity by disrupting cell membranes, inhibiting QS, and targeting the EPS matrix (Kotrange et al., 2021). For example, silver nanoparticles significantly reduce the colonization of bacteria such as *S. aureus, E. coli,* and *K. pneumoniae* on catheter surfaces. Similarly, ZnO and Cu nanoparticles inhibit fungal biofilms, offering alternative therapeutic options (Joshi et al., 2022).

7.3. Quorum Sensing Inhibition

Quorum sensing inhibition is an innovative strategy that targets bacterial communication to prevent biofilm formation (Zhao et al., 2020). These inhibitors reduce bacterial virulence factors and limit the development of antibiotic resistance by preventing the production of autoinducer molecules (Naga et al., 2023). Additionally, bacteriophage-based inhibitors disrupt bacterial signaling pathways, helping to control infections and providing a more sustainable solution against the development of resistance (Faleiro et al., 2022).

7.4. Enzymatic Degradation of Biofilms

Enzymatic treatments target the biofilm matrix, facilitating its breakdown and removal. Enzymes such as proteases, lipases, amylases, and DNases degrade key structural components of the biofilm, rendering microorganisms more susceptible to antibiotics (Pakkulnan et al., 2023).

Enzymes such as lysozyme and Dispersin B support biofilm elimination by targeting the cell wall and EPS structure. However, factors such as high cost, environmental sensitivity, and the risk of surface damage limit their effectiveness (Amankwah et al., 2021).

7.5. Antimicrobial Photodynamic and Sonodynamic Therapy

Photodynamic (aPDT) and sonodynamic (aSDT) therapies eliminate bacteria through reactive oxygen species generated upon activation by light or ultrasound energy. While aPDT is suitable for superficial biofilms, aSDT is effective in infections located in deeper tissues. Both approaches provide safe and non-invasive options against antibiotic-resistant bacteria (Garapati et al., 2023; Xu et al., 2023).

The combination of aPDT with PNA nanoparticles enhances treatment efficacy by allowing deeper penetration into the biofilm (Farahani et al., 2021). The use of ultrasound in combination with antibiotics enhances biofilm disruption and drug penetration. The combined application of these two approaches provides a synergistic effect in the treatment of biofilm-associated infections (Xiu et al., 2023).

CONCLUSION

Bacterial biofilm formation is a multi-stage process involving microbial cell adhesion to a surface, EPS synthesis, and community-level organization. This process plays a critical role in enabling bacteria to develop resistance against antibiotics, host immune responses, and environmental stresses. Clinically, biofilm-forming microorganisms are responsible for a range of infections, including those associated with medical devices, chronic wound infections, and catheters, complicating treatment and increasing the risk of recurrence. Compared to planktonic bacteria, cells within biofilms exhibit significantly higher levels of resistance due to reduced antibiotic penetration, efflux systems, target modification, metabolically inactive "persister" cells, and horizontal gene transfer.

Traditional approaches for managing biofilm associated infections such as mechanical cleaning, surface modifications, chemical disinfectants, and antimicrobial agents remain important but demonstrate limited efficacy against mature biofilm communities. Consequently, there is a need for novel strategies that disrupt biofilm structures or prevent their formation.

In this context, emerging therapeutic options including natural compounds, nanotechnology-based approaches, quorum sensing inhibitors, enzymatic degradation, and antimicrobial therapies have become areas of active research. These methods exhibit effects such as disrupting biofilm architecture, resensitizing bacteria to antibiotics, suppressing virulence factors, and ultimately supporting and enhancing treatment outcomes.

In conclusion, biofilm formation is not merely a biological system facilitating microbial survival; it also represents a clinical challenge due to its contribution to antimicrobial resistance, chronic infections, and treatment failures. Therefore, a deeper understanding of biofilm formation mechanisms, the development of early diagnostic tools, and the widespread implementation of biofilm-targeted therapeutic strategies are of great importance for future infection control and the management of resistant microbial pathogens.

REFERENCES

Alexander, S. A., & Schiesser, C. H. (2016). Heteroorganic molecules and bacterial biofilms: Controlling biodeterioration of cultural heritage. In Arkivoc (Vol. 2017, Issue 2, pp. 180–222). Arkat. doi: 10.3998/ark.5550190.p009.765

Allegrone, G., Ceresa, C., Rinaldi, M., & Fracchia, L. (2021). Diverse effects of natural and synthetic surfactants on the inhibition of staphylococcus aureus biofilm. *Pharmaceutics*, *13*(8). doi: 10.3390/pharmaceutics13081172

Allkja, J., Van Charante, F., Aizawa, J., Reigada, I., Guarch-Pérez, C., Vazquez-Rodriguez, J. A., Cos, P., Coenye, T., Fallarero, A., Zaat, S., Felici, A., Ferrari, L., Azevedo, N., Parker, A., & Goeres, D. (2021). Interlaboratory study for the evaluation of three microtiter plate-based biofilm quantification methods. *Scientific Reports*, *11*. doi: 10.1038/s41598-021-93115-w

Altuwaijri, N., Fitaihi, R., Alkathiri, F., Bukhari, S., Altalal, A., Alsalhi, A., Alsulaiman, L., Alomran, A., Aldosari, N., Alqhafi, S., Alhamdan, M., & Alfaraj, R. (2025). Assessing the Antibacterial Potential and Biofilm Inhibition Capability of Atorvastatin-Loaded Nanostructured Lipid Carriers via Crystal Violet Assay. *Pharmaceuticals*, *18*. doi: 10.3390/ph18030417

Amador, C., Stannius, R. O., Røder, H., & Burmølle, M. (2021). High-throughput screening alternative to crystal violet biofilm assay combining fluorescence quantification and imaging. *Journal of Microbiological Methods*, 106343. doi: 10.1016/j.mimet.2021.106343

Amankwah, S., Abdella, K., & Kassa, T. (2021). Bacterial biofilm destruction: A focused review on the recent use of phage-based strategies with other antibiofilm agents. In Nanotechnology, Science and Applications (Vol. 14, pp. 161–177). Dove Medical Press Ltd. doi: 10.2147/NSA.S325594

Amod, A., Anand, A. A., Sahoo, A., & Samanta, S. (2025). Diagnostic and therapeutic strategies in combating implanted medical device-associated bacterial biofilm infections. *Folia Microbiologica*. doi: 10.1007/s12223-025-01242-y

Anan, M., Abu-El-Azayem, A., & Elkashef, S. (2024). Comparison of two invitro phenotypic methods (Tissue Culture plate and Congo Red Agar) for Detection of Biofilm Formation by Enterococci. *Egyptian Journal of Medical Microbiology*. doi: 10.21608/ejmm.2024.325935

Andersen, J. B., Rybtke, M., & Tolker-Nielsen, T. (2024). The dynamics of biofilm development and dispersal should be taken into account when quantifying biofilm via the crystal violet microtiter plate assay. *Biofilm*, *8*. doi: 10.1016/j.bioflm.2024.100207

Anderson, J. K., Huang, J. Y., Wreden, C., Sweeney, E. G., Goers, J., James Remington, S., & Guillemin, K. (2015). Chemorepulsion from the quorum signal autoinducer-2 promotes Helicobacter pylori biofilm dispersal. *MBio*, *6*(4). doi: 10.1128/mBio.00379-15

Anju, V. T., Busi, S., Imchen, M., Kumavath, R., Mohan, M. S., Salim, S. A., Subhaswaraj, P., & Dyavaiah, M. (2022). Polymicrobial Infections and Biofilms: Clinical Significance and Eradication Strategies. In Antibiotics (Vol. 11, Issue 12). MDPI. doi: 10.3390/antibiotics11121731

Bahuguna, A., Khan, I., Bajpai, V., & Kang, S. (2017). MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh Journal of Pharmacology*, *12*, 8–2017. doi: 10.3329/bjp.v12i2.30892

Bai, Y. Bin, Shi, M. Y., Wang, W. W., Wu, L. Y., Bai, Y. T., Li, B., Zhou, X. Z., & Zhang, J. Y. (2022). Novel quorum sensing inhibitor Echinatin as an antibacterial synergist against Escherichia coli. *Frontiers in Microbiology*, *13*. doi: 10.3389/fmicb.2022.1003692

Basnet, A., Tamang, B., Shrestha, M., Shrestha, L., Rai, J., Maharjan, R., Dahal, S., Shrestha, P., & Rai, S. (2023). Assessment of four in vitro phenotypic biofilm detection methods in relation to antimicrobial resistance in aerobic clinical bacterial isolates. *PLOS ONE*, *18*. doi: 10.1371/journal.pone.0294646

Batoni, G., Martínez-Pomares, L., & Esin, S. (2021). Editorial: Immune Response to Biofilms. *Frontiers in Immunology*, *12*. doi: 10.3389/fimmu.2021.696356

Bayramov, D. F., & Neff, J. A. (2017). Beyond conventional antibiotics — New directions for combination products to combat biofilm.

Advanced Drug Delivery Reviews, *112*, 48–60. doi: 10.1016/J.ADDR.2016.07.010

Böhning, J., Tarafder, A. K., & Bharat, T. A. M. (2024). The role of filamentous matrix molecules in shaping the architecture and emergent properties of bacterial biofilms. In Biochemical Journal (Vol. 481, Issue 4, pp. 245–263). Portland Press Ltd. doi: 10.1042/BCJ20210301

Bouhrour, N., Nibbering, P., & Bendali, F. (2024). Medical Device-Associated Biofilm Infections and Multidrug-Resistant Pathogens. *Pathogens*, *13*. doi: 10.3390/pathogens13050393

Caldara, M., Belgiovine, C., Secchi, E., & Rusconi, R. (2022). Environmental, Microbiological, and Immunological Features of Bacterial Biofilms Associated with Implanted Medical Devices. *Clinical Microbiology Reviews*, *35*. doi: 10.1128/cmr.00221-20

Campoccia, D., Montanaro, L., & Arciola, C. (2021). Extracellular DNA (eDNA). A Major Ubiquitous Element of the Bacterial Biofilm Architecture. *International Journal of Molecular Sciences*, *22*. doi: 10.3390/ijms22169100

Cangui-Panchi, S. P., Ñacato-Toapanta, A. L., Enríquez-Martínez, L. J., Salinas-Delgado, G. A., Reyes, J., Garzon-Chavez, D., & Machado, A. (2023). Battle royale: Immune response on biofilms - host-pathogen interactions. *Current Research in Immunology*, *4*, 100057. doi: 10.1016/j.crimmu.2023.100057

CDC. (2019). *Centers for Disease Control and Prevention: antibiotic resistance threats in the United States, Atlanta, GA*. Retrieved from https://ndc.services.cdc.gov/wp-content/uploads/Antibiotic-Resistance-Threats-in-the-United-States-2019.pdf

Centorame, P., Iacone, L., Salini, R., Ciarulli, A., Guidi, F., & Pomilio, F. (2020). Biofilm production by Listeria monocytogenes strains: detection with colorimetric analysis. *European Journal of Public Health*, *30*. doi: 10.1093/eurpub/ckaa166.238

Chavez-Dozal, A., Nourabadi, N., Erken, M., McDougald, D., & Nishiguchi, M. (2016). Comparative analysis of quantitative methodologies for Vibrionaceae biofilms. *Folia Microbiologica*, *61*, 449–453. doi: 10.1007/s12223-016-0456-9

Cleaver, L., & Garnett, J. A. (2023). How to study biofilms: technological advancements in clinical biofilm research. In Frontiers in Cellular and Infection Microbiology (Vol. 13). Frontiers Media SA. doi: 10.3389/fcimb.2023.1335389

Corte, L., Pierantoni, C., Tascini, C., Roscini, L., & Cardinali, G. (2019). Biofilm Specific Activity: A Measure to Quantify Microbial Biofilm. *Microorganisms*, *7*. doi: 10.3390/microorganisms7030073

Cruickshank, D., Hamilton, D., Iloba, I., & Jensen, G. (2024). Secreted Metabolites from Pseudomonas, Staphylococcus, and Borrelia Biofilm: Modulation of Immunogenicity by a Nutraceutical Enzyme and Botanical Blend. *Microorganisms*, *12*. doi: 10.3390/microorganisms12050991

Darvishi, S., Tavakoli, S., Kharaziha, M., Girault, H. H., Kaminski, C. F., & Mela, I. (2022). Advances in the Sensing and Treatment of Wound Biofilms. In Angewandte Chemie - International Edition (Vol. 61, Issue 13). John Wiley and Sons Inc. doi: 10.1002/anie.202112218

De Jesus, R., & Dedeles, G. (2019). Data on quantitation of Bacillus cereus sensu lato biofilms by microtiter plate biofilm formation assay. *Data in Brief*, *28*. doi: 10.1016/j.dib.2019.104951

De Oliveira, D. M. P., Forde, B. M., Kidd, T. J., Harris, P. N. A., Schembri, M. A., Beatson, S. A., Paterson, D. L., & Walker, M. J. (2020). *Antimicrobial Resistance in ESKAPE Pathogens*. doi: 10.1128/CMR

Dekimpe, V., & Déziel, E. (2009). Revisiting the quorum-sensing hierarchy in Pseudomonas aeruginosa: The transcriptional regulator RhlR regulates LasR-specific factors. *Microbiology*, *155*(3), 712–723. doi: 10.1099/mic.0.022764-0

Delik, E., Eroğlu, B., Çolak, Ç. Y., Özçelik, A. T., & Tefon Öztürk, B. E. (2023). Alterations of growth, biofilm-forming, and gene expression of Bordetella pertussis by antibiotics at sub-minimum inhibitory concentrations. *Research in Microbiology*, *174*(5), 104058. doi: 10.1016/J.RESMIC.2023.104058

Desmond, P., Best, J., Morgenroth, E., & Derlon, N. (2017). Linking composition of extracellular polymeric substances (EPS) to the physical structure and hydraulic resistance of membrane biofilms. *Water Research*, *132*, 211–221. doi: 10.1016/j.watres.2017.12.058

Di Domenico, E., Oliva, A., & Guembe, M. (2022). The Current Knowledge on the Pathogenesis of Tissue and Medical Device-Related Biofilm Infections. *Microorganisms*, *10*. doi: 10.3390/microorganisms10071259

Diban, F., Di Lodovico, S., Di Fermo, P., D'Ercole, S., D'Arcangelo, S., Di Giulio, M., & Cellini, L. (2023). Biofilms in Chronic Wound Infections: Innovative Antimicrobial Approaches Using the In Vitro Lubbock Chronic Wound Biofilm Model. In International Journal of Molecular Sciences (Vol. 24, Issue 2). MDPI. doi: 10.3390/ijms24021004

Dogan, O., Atac, N., Babuççu, G., & Can, F. (2021). Comparison of Crystal Violet Staining Assay and XTT Methods in the Evaluation of Biofilm Formation in Candida parapsilosis Candidemia Isolates. *Infectious Diseases and Clinical Microbiology*. doi: 10.36519/idcm.2021.104

Er, S. (2024). COMPARISON OF THE MICROTITER PLATE METHOD AND THE CONGO RED AGAR TECHNIQUE IN THE DETERMINATION OF STAPHYLOCOCCAL BIOFILM. *Mugla Journal of Science and Technology*.
doi: 10.22531/muglajsci.1493167

Fagerlund, A., Heir, E., Møretrø, T., & Langsrud, S. (2020). Listeria monocytogenes biofilm removal using different commercial cleaning agents. *Molecules*, *25*(4). doi: 10.3390/molecules25040792

Faleiro, L., Marques, A., Martins, J., Jordão, L., Nogueira, I., Gumerova, N. I., Rompel, A., & Aureliano, M. (2022). The Preyssler-Type Polyoxotungstate Exhibits Anti-Quorum Sensing, Antibiofilm, and Antiviral Activities. *Biology*, *11*(7). doi: 10.3390/biology11070994

Fang, K., Park, O. J., & Hong, S. H. (2020). Controlling biofilms using synthetic biology approaches. *Biotechnology Advances*, *40*, 107518. doi: 10.1016/J.BIOTECHADV.2020.107518

Farahani, N. N., Kalani, B. S., Monavari, S. H., Mirkalantari, S., Montazer, F., Sholeh, M., Javanmard, Z., & Irajian, G. (2021). *Therapeutic effects, immunogenicity and cytotoxicity of a cell penetrating peptide-peptide nucleic acid conjugate against cagA of Helicobacter pylori in cell culture and animal model* (Vol. 13, Issue 3). Retrieved from http://ijm.tums.ac.ir

Flemming, H., Van Hullebusch, E., Little, B., Neu, T., Nielsen, P., Seviour, T., Stoodley, P., Wingender, J., & Wuertz, S. (2024). Microbial extracellular polymeric substances in the environment, technology and medicine. *Nature Reviews. Microbiology*. doi: 10.1038/s41579-024-01098-y

Flemming, H., Van Hullebusch, E., Neu, T., Nielsen, P., Seviour, T., Stoodley, P., Wingender, J., & Wuertz, S. (2022). The biofilm matrix: multitasking in a shared space. *Nature Reviews Microbiology*, *21*, 70–86. doi: 10.1038/s41579-022-00791-0

Funari, R., & Shen, A. Q. (2022). Detection and Characterization of Bacterial Biofilms and Biofilm-Based Sensors. In ACS Sensors (Vol. 7, Issue 2, pp. 347–357). American Chemical Society. doi: 10.1021/acssensors.1c02722

Furtuna, D. K., Debora, K., & Warsito, E. (2018). *Comparison of Microbiological Examination by Test Tube and Congo Red Agar Methods to Detect Biofilm Production on Clinical Isolates*. *54*, 22–28. doi: 10.20473/fmi.v54i1.8047

Gangashettappa, N., Raksha, L., Shantala, G., Nandan, B., & Sinha, D. (2019). Study of biofilm formation in bacterial isolates from contact lens wearers. *Indian Journal of Ophthalmology*, *68*, 23–28. doi: 10.4103/ijo.ijo_947_19

Ge, M., Zhu, W., Mei, J., Hu, T., Yang, C., Lin, H., & Shi, J. (2024). Piezoelectric-Enhanced Nanocatalysts Trigger Neutrophil N1 Polarization against Bacterial Biofilm by Disrupting Redox Homeostasis. *Advanced Materials*, *37*. doi: 10.1002/adma.202409633

George, E. A., & Muir, T. W. (2007). Molecular mechanisms of agr quorum sensing in virulent staphylococci. In ChemBioChem (Vol. 8, Issue 8, pp. 847–855). doi: 10.1002/cbic.200700023

George, T., Sivam, V., Vaiyapuri, M., Anandan, R., Sivaraman, G., & Joseph, T. (2025). Standardizing biofilm quantification:

harmonizing crystal violet absorbance measurements through extinction coefficient ratio adjustment. *Archives of Microbiology*, *207 3*, 59. doi: 10.1007/s00203-025-04251-0

Ghasemi, M., Liang, S., Luu, Q., & Kempson, I. (2023). The MTT Assay: A Method for Error Minimization and Interpretation in Measuring Cytotoxicity and Estimating Cell Viability. *Methods in Molecular Biology*, *2644*, 15–33. doi: 10.1007/978-1-0716-3052-5_2

Gobor, T., Corol, G., Ferreira, L., Rymovicz, A., Rosa, R., Campelo, P., & Rosa, E. (2011). Proposal of protocols using D-glutamine to optimize the 2,3-bis(2-methoxy-4-nitro-5-sulfophenly)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) assay for indirect estimation of microbial loads in biofilms of medical importance. *Journal of Microbiological Methods*, *84 2*, 299–306. doi: 10.1016/j.mimet.2010.12.018

Halawa, E. M., Fadel, M., Al-Rabia, M. W., Behairy, A., Nouh, N. A., Abdo, M., Olga, R., Fericean, L., Atwa, A. M., El-Nablaway, M., & Abdeen, A. (2023). Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. In Frontiers in Pharmacology (Vol. 14). Frontiers Media SA. doi: 10.3389/fphar.2023.1305294

Halim, R. A., Kassem, N., & Mahmoud, B. (2018). Detection of Biofilm Producing Staphylococci among Different Clinical Isolates and Its Relation to Methicillin Susceptibility. *Open Access Macedonian*

Journal of Medical Sciences, *6*, 1335–1341. doi: 10.3889/oamjms.2018.246

Haney, E. F., Trimble, M. J., Cheng, J. T., Vallé, Q., & Hancock, R. E. W. (2018). Critical assessment of methods to quantify biofilm growth and evaluate antibiofilm activity of host defence peptides. *Biomolecules*, *8*(2). doi: 10.3390/biom8020029

Harika, K., Shenoy, V., Narasimhaswamy, N., & Chawla, K. (2020). Detection of Biofilm Production and Its Impact on Antibiotic Resistance Profile of Bacterial Isolates from Chronic Wound Infections. *Journal of Global Infectious Diseases*, *12*, 129–134. doi: 10.4103/jgid.jgid_150_19

Hasan, M. I., & Aggarwal, S. (2024). Matrix matters: How extracellular substances shape biofilm structure and mechanical properties. *Colloids and Surfaces. B, Biointerfaces*, *246*, 114341. doi: 10.1016/j.colsurfb.2024.114341

Hemmati, F., Rezaee, M. A., Ebrahimzadeh, S., Yousefi, L., Nouri, R., Kafil, H. S., & Gholizadeh, P. (2021). Novel Strategies to Combat Bacterial Biofilms. In Molecular Biotechnology (Vol. 63, Issue 7, pp. 569–586). Humana Press Inc. doi: 10.1007/s12033-021-00325-8

Hochma, E., Yarmolinsky, L., Khalfin, B., Nisnevitch, M., Ben-Shabat, S., & Nakonechny, F. (2021). Antimicrobial Effect of Phytochemicals from Edible Plants. In Processes (Vol. 9, Issue 11). MDPI. doi: 10.3390/PR9112089

Hoogstraten, C., Smeitink, J., Russel, F., & Schirris, T. (2022). Dissecting Drug-Induced Cytotoxicity and Metabolic Dysfunction in Conditionally Immortalized Human Proximal Tubule Cells. *Frontiers in Toxicology*, *4*. doi: 10.3389/ftox.2022.842396

Jamuna Bai, A., & Ravishankar Rai, V. (2016). Effect of small chain N acyl homoserine lactone quorum sensing signals on biofilms of food-borne pathogens. *Journal of Food Science and Technology*, *53*(9), 3609–3614. doi: 10.1007/s13197-016-2346-1

Jiang, Q., Chen, J., Yang, C., Yin, Y., Yao, K., & Song, D. (2019). Quorum Sensing: A Prospective Therapeutic Target for Bacterial Diseases. In BioMed Research International (Vol. 2019). Hindawi Limited. doi: 10.1155/2019/2015978

Joshi, K. M., Shelar, A., Kasabe, U., Nikam, L. K., Pawar, R. A., Sangshetti, J., Kale, B. B., Singh, A. V., Patil, R., & Chaskar, M. G. (2022). Biofilm inhibition in Candida albicans with biogenic hierarchical zinc-oxide nanoparticles. *Biomaterials Advances*, *134*, 112592. doi: 10.1016/J.MSEC.2021.112592

Kameswaran, S., Gujjala, S., Zhang, S., Kondeti, S., Mahalingam, S., Bangeppagari, M., & Bellemkonda, R. (2024). Quenching and quorum sensing in bacterial bio-films. *Research in Microbiology*, *175*(3). doi: 10.1016/j.resmic.2023.104085

Kamimura, R., Kanematsu, H., Ogawa, A., Kogo, T., Miura, H., Kawai, R., Hirai, N., Kato, T., Yoshitake, M., & Barry, D. (2022).

Quantitative Analyses of Biofilm by Using Crystal Violet Staining and Optical Reflection. *Materials*, *15*. doi: 10.3390/ma15196727

Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. (2020). Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. *Trends in Microbiology*, *28 8*, 668–681. doi: 10.1016/j.tim.2020.03.016

Kavanaugh, J. S., Flack, C. E., Lister, J., Ricker, E. B., Ibberson, C. B., Jenul, C., Moormeier, D. E., Delmain, E. A., Bayles, K. W., & Horswill, A. R. (2019). Identification of extracellular DNA-binding proteins in the biofilm matrix. *MBio*, *10*(3). doi: 10.1128/mBio.01137-19

Khan, A. A., Manzoor, K. N., Sultan, A., Saeed, M., Rafique, M., Noushad, S., Talib, A., Rentschler, S., & Deigner, H. P. (2021). Pulling the brakes on fast and furious multiple drug-resistant (MDR) bacteria. *International Journal of Molecular Sciences*, *22*(2), 1–40. doi: 10.3390/ijms22020859

Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T.-F., Alarcon, E. I., & Alarcon Bacterial, E. I. (2018). Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*, *4*, e01067. doi: 10.1016/j.heliyon.2018

Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P., & Hall-Stoodley, L. (2017). Targeting microbial biofilms: Current and prospective therapeutic strategies. In Nature Reviews Microbiology (Vol. 15, Issue 12, pp. 740–755). Nature Publishing Group. doi: 10.1038/nrmicro.2017.99

Kord, M., Ardebili, A., Jamalan, M., Jahanbakhsh, R., Behnampour, N., & Ghaemi, E. (2018). Evaluation of Biofilm Formation and Presence of Ica Genes in Staphylococcus epidermidis Clinical Isolates. *Osong Public Health and Research Perspectives*, *9*, 160–166. doi: 10.24171/j.phrp.2018.9.4.04

Kostoglou, D., Protopappas, I., & Giaouris, E. (2020). Common plant-derived terpenoids present increased anti-biofilm potential against staphylococcus bacteria compared to a quaternary ammonium biocide. *Foods*, *9*(6). doi: 10.3390/foods9060697

Kotrange, H., Najda, A., Bains, A., Gruszecki, R., Chawla, P., Tosif, M. M., & Thakur, K. (2021). Molecular Sciences Metal and Metal Oxide Nanoparticle as a Novel Antibiotic Carrier for the Direct Delivery of Antibiotics. *J. Mol. Sci*, 22. doi: 10.3390/ijms

Kragh, K., Alhede, M., Kvich, L., & Bjarnsholt, T. (2019). Into the well—A close look at the complex structures of a microtiter biofilm and the crystal violet assay. *Biofilm*, *1*. doi: 10.1016/j.bioflm.2019.100006

Kumar, L., Bisen, M., Harjai, K., Chhibber, S., Azizov, S., Lalhlenmawia, H., & Kumar, D. (2023). Advances in Nanotechnology for Biofilm Inhibition. In ACS Omega (Vol. 8, Issue 24, pp. 21391–21409). American Chemical Society. doi: 10.1021/acsomega.3c02239

Kumar, P., Nagarajan, A., & Uchil, P. (2018). Analysis of Cell Viability by the MTT Assay. *Cold Spring Harbor Protocols*, *2018 6*. doi: 10.1101/pdb.prot095505

Le, K., Park, M., & Otto, M. (2018). Immune Evasion Mechanisms of Staphylococcus epidermidis Biofilm Infection. *Frontiers in Microbiology*, *9*. doi: 10.3389/fmicb.2018.00359

Li, J., Liu, D., Tian, X., Koseki, S., Chen, S., Ye, X., & Ding, T. (2019). Novel antibacterial modalities against methicillin resistant Staphylococcus aureus derived from plants. In Critical Reviews in Food Science and Nutrition (Vol. 59, Issue S1, pp. S153–S161). Taylor and Francis Ltd. doi: 10.1080/10408398.2018.1541865

Li, P., Yin, R., Cheng, J., & Lin, J. (2023). Bacterial Biofilm Formation on Biomaterials and Approaches to Its Treatment and Prevention. *International Journal of Molecular Sciences*, *24*. doi: 10.3390/ijms241411680

Li, Y., Wang, S., Ding, H., Xiao, K., & Huang, X. (2023). Quorum sensing-Fe metabolism interplay affects biofouling on reverse osmosis membrane: Evidences from microbial shift and structure alteration. *Desalination*, *551*, 116416. doi: 10.1016/J.DESAL.2023.116416

Liu, S., Lu, H., Zhang, S., Shi, Y., & Chen, Q. (2022). Phages against Pathogenic Bacterial Biofilms and Biofilm-Based Infections: A Review. In Pharmaceutics (Vol. 14, Issue 2). MDPI. doi: 10.3390/pharmaceutics14020427

Magaña-Montiel, N., Muriel-Millán, L., & Pardo-López, L. (2024). XTT assay for detection of bacterial metabolic activity in water-based polyester polyurethane. *PLOS ONE*, *19*. doi: 10.1371/journal.pone.0303210

Malinowski, P., Skała, K., Jabłońska-Trypuć, A., Koronkiewicz, A., Wołejko, E., Wydro, U., Świderski, G., & Lewandowski, W. (2022). Comparison of the Usefulness of MTT and CellTiterGlo Tests Applied for Cytotoxicity Evaluation of Compounds from the Group of Polyphenols. *Innovations-Sustainability-Modernity-Openness Conference (ISMO&Rsquo;22)*. doi: 10.3390/environsciproc2022018009

Masters, E. A., Trombetta, R. P., de Mesy Bentley, K. L., Boyce, B. F., Gill, A. L., Gill, S. R., Nishitani, K., Ishikawa, M., Morita, Y., Ito, H., Bello-Irizarry, S. N., Ninomiya, M., Brodell, J. D., Lee, C. C., Hao, S. P., Oh, I., Xie, C., Awad, H. A., Daiss, J. L., … Muthukrishnan, G. (2019). Evolving concepts in bone infection: redefining "biofilm", "acute vs. chronic osteomyelitis", "the immune proteome" and "local antibiotic therapy." In Bone Research (Vol. 7, Issue 1). Sichuan University. doi: 10.1038/s41413-019-0061-z

Mathew, B., Gupta, P., Naaz, T., Rai, R., Gupta, S., Gupta, S., Chaurasiya, S., Purwar, S., Biswas, D., Vyas, A., & Singh, A. (2023). Role of Streptococcus pneumoniae extracellular glycosidases in immune evasion. *Frontiers in Cellular and Infection Microbiology*, *13*. doi: 10.3389/fcimb.2023.1109449

Mendhe, S., Badge, A., Ugemuge, S., & Chandi, D. (2023). Impact of Biofilms on Chronic Infections and Medical Challenges. *Cureus*. doi: 10.7759/cureus.48204

Michaelis, C., & Grohmann, E. (2023). Horizontal Gene Transfer of Antibiotic Resistance Genes in Biofilms. In Antibiotics (Vol. 12, Issue 2). MDPI. doi: 10.3390/antibiotics12020328

Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S., & Rijal, G. (2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. In AIMS Microbiology (Vol. 8, Issue 3, pp. 240–278). AIMS Press. doi: 10.3934/microbiol.2022019

Mirzaei, R., Sabokroo, N., Ahmadyousefi, Y., Motamedi, H., & Karampoor, S. (2022). Immunometabolism in biofilm infection: lessons from cancer. *Molecular Medicine*, *28*. doi: 10.1186/s10020-022-00435-2

Mishra, A., Aggarwal, A., & Khan, F. (2024). Medical Device-Associated Infections Caused by Biofilm-Forming Microbial Pathogens and Controlling Strategies. *Antibiotics*, *13*. doi: 10.3390/antibiotics13070623

Moreau, A., Nguyen, D., Hinbest, A., Zamora, A., Weerasekera, R., Matej, K., Zhou, X., Sanchez, S., Brenes, I. R., Tai, J.-S. B., Nadell, C., Ng, W.-L., Gordon, V., Komarova, N., Olson, R., Li, Y., & Yan, J. (2025). Surface remodeling and inversion of cell-matrix interactions underlie community recognition and dispersal in Vibrio cholerae biofilms. *Nature Communications*, *16*. doi: 10.1038/s41467-024-55602-2

Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X., & Huang, T. (2020). Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. In Frontiers in Microbiology (Vol. 11). Frontiers Media S.A. doi: 10.3389/fmicb.2020.00928

Naga, N. G., El-Badan, D. E., Ghanem, K. M., & Shaaban, M. I. (2023). It is the time for quorum sensing inhibition as alternative strategy of antimicrobial therapy. In Cell Communication and Signaling (Vol. 21, Issue 1). BioMed Central Ltd. doi: 10.1186/s12964-023-01154-9

Nuță, D. C., Limban, C., Chiriță, C., Chifiriuc, M. C., Costea, T., Ioniță, P., Nicolau, I., & Zarafu, I. (2021). Contribution of essential oils to the fight against microbial biofilms—a review. In Processes (Vol. 9, Issue 3). MDPI AG. doi: 10.3390/pr9030537

Ommen, P., Zobek, N., & Meyer, R. (2017). Quantification of biofilm biomass by staining: Non-toxic safranin can replace the popular crystal violet. *Journal of Microbiological Methods*, *141*, 87–89. doi: 10.1016/j.mimet.2017.08.003

Omwenga, Eric O, Awour, S. O., & Goycoolea, F. M. (2023). Paradigm shift: Bacterial quorum sensing and possible control targets. *Journal of Clinical Images and Medical Case Reports*. doi: 10.52768/2766-7820/2632

Omwenga, Eric Omori, & Awuor, S. O. (2024). The Bacterial Biofilms: Formation, Impacts, and Possible Management Targets in the Healthcare System. In Canadian Journal of Infectious Diseases and Medical Microbiology (Vol. 2024, Issue 1). John Wiley and Sons Ltd. doi: 10.1155/cjid/1542576

Pakkulnan, R., Thonglao, N., & Chareonsudjai, S. (2023). DNase I and chitosan enhance efficacy of ceftazidime to eradicate Burkholderia pseudomallei biofilm cells. *Scientific Reports*, *13*(1). doi: 10.1038/s41598-023-27790-2

Pandit, S., Fazilati, M., Gaska, K., Derouiche, A., Nypelö, T., Mijakovic, I., & Kádár, R. (2020). The Exo-Polysaccharide Component of Extracellular Matrix is Essential for the Viscoelastic Properties of Bacillus subtilis Biofilms. *International Journal of Molecular Sciences*, *21*. doi: 10.3390/ijms21186755

Peng, Q., Tang, X., Dong, W., Sun, N., & Yuan, W. (2022). A Review of Biofilm Formation of Staphylococcus aureus and Its Regulation Mechanism. *Antibiotics*, *12*. doi: 10.3390/antibiotics12010012

Peng, Q., Tang, X., Dong, W., Zhi, Z., Zhong, T., Lin, S., Ye, J., Qian, X., Chen, F., & Yuan, W. (2023). Carvacrol inhibits bacterial polysaccharide intracellular adhesin synthesis and biofilm formation of mucoid Staphylococcus aureus: an in vitro and in vivo study. *RSC Advances*, *13*(41), 28743–28752. doi: 10.1039/d3ra02711b

Percival, S., Suleman, L., Vuotto, C., & Donelli, G. (2015). Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *Journal of Medical Microbiology*, *64 Pt 4*, 323–334. doi: 10.1099/jmm.0.000032

Pourhajibagher, M., Pourakbari, B., & Bahador, A. (2022). Contribution of antimicrobial photo-sonodynamic therapy in wound healing: an in vivo effect of curcumin-nisin-based poly (L-lactic acid) nanoparticle on Acinetobacter baumannii biofilms. *BMC Microbiology*, *22*(1). doi: 10.1186/s12866-022-02438-9

Preda, V. G., & Săndulescu, O. (2019). Communication is the key: biofilms, quorum sensing, formation and prevention. *Discoveries*, *7*(3), e10. doi: 10.15190/d.2019.13

Ramage, G. (2016). Comparing apples and oranges: considerations for quantifying candidal biofilms with XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide] and the need for standardized testing. *Journal of Medical Microbiology*, *65 4*, 259–260. doi: 10.1099/jmm.0.000237

Ramakrishnan, R., Singh, A. K., Singh, S., Chakravortty, D., & Das, D. (2022). Enzymatic dispersion of biofilms: An emerging biocatalytic avenue to combat biofilm-mediated microbial infections. In Journal of Biological Chemistry (Vol. 298, Issue 9). American Society for Biochemistry and Molecular Biology Inc. doi: 10.1016/j.jbc.2022.102352

Ramírez-Larrota, J. S., & Eckhard, U. (2022). An Introduction to Bacterial Biofilms and Their Proteases, and Their Roles in Host

Infection and Immune Evasion. In Biomolecules (Vol. 12, Issue 2). MDPI. doi: 10.3390/biom12020306

Rather, M. A., Gupta, K., & Mandal, M. (2021). Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. In Brazilian Journal of Microbiology (Vol. 52, Issue 4, pp. 1701–1718). Springer Science and Business Media Deutschland GmbH. doi: 10.1007/s42770-021-00624-x

Sabzi, S., Habibi, M., Badmasti, F., Shahbazi, S., Reza, M., Karam, A., & Farokhi, M. (2024). *Polydopamine-based nano adjuvant as a promising vaccine carrier induces significant immune responses against Acinetobacter baumannii- associated pneumonia*.

Saharan, B. S., Beniwal, N., & Duhan, J. S. (2024). From formulation to function: A detailed review of microbial biofilms and their polymer-based extracellular substances. *The Microbe*, *5*, 100194. doi: 10.1016/J.MICROB.2024.100194

Sahoo, K., & Meshram, S. (2024). Biofilm Formation in Chronic Infections: A Comprehensive Review of Pathogenesis, Clinical Implications, and Novel Therapeutic Approaches. *Cureus*. doi: 10.7759/cureus.70629

Sahu, A., & Ruhal, R. (2025). Immune system dynamics in response to Pseudomonas aeruginosa biofilms. *NPJ Biofilms and Microbiomes*, *11*. doi: 10.1038/s41522-025-00738-2

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle:

expanding the conceptual model of biofilm formation. In Nature Reviews Microbiology (Vol. 20, Issue 10, pp. 608–620). Nature Research. doi: 10.1038/s41579-022-00767-0

Scalia, A., & Najmi, Z. (2025). Targeting Bacterial Biofilms on Medical Implants: Current and Emerging Approaches. *Antibiotics*. doi: 10.3390/antibiotics14080802

Schilcher, K., & Horswill, A. R. (2020). *Staphylococcal Biofilm Development: Structure, Regulation, and Treatment Strategies*. Retrieved from https://doi.org/10

Schulze, A., Mitterer, F., Pombo, J. P., & Schild, S. (2021). Biofilms by bacterial human pathogens: Clinical relevance - Development, composition and regulation - Therapeutical strategies. *Microbial Cell*, *8*(2), 28–56. doi: 10.15698/MIC2021.02.741

Secchi, E. ;, Savorana, G. ;, Vitale, A. ;, Eberl, L. ;, Stocker, R. ;, & Rusconi, R. (2022). *The structural role of bacterial eDNA in the formation of biofilm streamers Rights / license: Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Funding acknowledgement:-The role of ambient flow and physico-chemical microenvironment in determining the microstructure of the biofilm matrix ()*. doi: 10.3929/ethz-b-000540023

Sharma, D., Misba, L., & Khan, A. U. (2019). Antibiotics versus biofilm: An emerging battleground in microbial communities. In Antimicrobial Resistance and Infection Control (Vol. 8, Issue 1). BioMed Central Ltd. doi: 10.1186/s13756-019-0533-3

Sharma, S., Mohler, J., Mahajan, S., Schwartz, S., Bruggemann, L., & Aalinkeel, R. (2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms*, *11*. doi: 10.3390/microorganisms11061614

Sheridan, M., Winters, C., Zamboni, F., & Collins, M. N. (2022). Biomaterials: Antimicrobial surfaces in biomedical engineering and healthcare. *Current Opinion in Biomedical Engineering*, *22*, 100373. doi: 10.1016/J.COBME.2022.100373

Shukla, S., & Rao, T. (2017). An Improved Crystal Violet Assay for Biofilm Quantification in 96-Well Microtitre Plate. *BioRxiv*. doi: 10.1101/100214

Stiefel, P., Rosenberg, U., Schneider, J., Mauerhofer, S., Maniura-Weber, K., & Ren, Q. (2016). Is biofilm removal properly assessed? Comparison of different quantification methods in a 96-well plate system. *Applied Microbiology and Biotechnology*, *100*, 4135–4145. doi: 10.1007/s00253-016-7396-9

Stindlova, M., Peroutka, V., Zdeňková, K., & Lencova, S. (2025). Assessing metabolic activity of yeast biofilm-forming cells on nanofibrous materials using MTT assay. *Folia Microbiologica*. doi: 10.1007/s12223-025-01304-1

Stoodley, P., Sauer, K., Davies, D. G., & Costerton, J. W. (2002). Biofilms as complex differentiated communities. In Annual Review of Microbiology (Vol. 56, pp. 187–209). doi: 10.1146/annurev.micro.56.012302.160705

String, G., Domini, M., Mirindi, P., Brodsky, H., Kamal, Y., Tatro, T., Johnston, M., Badr, H., & Lantagne, D. (2020). Efficacy of locally-available cleaning methods in removing biofilms from taps and surfaces of household water storage containers. *Npj Clean Water*, *3*(1). doi: 10.1038/s41545-020-0061-y

Su, Y., Yrastorza, J. T., Matis, M., Cusick, J., Zhao, S., Wang, G., & Xie, J. (2022). Biofilms: Formation, Research Models, Potential Targets, and Methods for Prevention and Treatment. In Advanced Science (Vol. 9, Issue 29). John Wiley and Sons Inc. doi: 10.1002/advs.202203291

Thibeaux, R., Kainiu, M., & Goarant, C. (2020). Biofilm Formation and Quantification Using the 96-Microtiter Plate. *Methods in Molecular Biology*, *2134*, 207–214. doi: 10.1007/978-1-0716-0459-5_19

Thomsen, K., Høiby, N., Jensen, P., Ciofu, O., & Moser, C. (2022). Immune Response to Biofilm Growing Pulmonary Pseudomonas aeruginosa Infection. *Biomedicines*, *10*. doi: 10.3390/biomedicines10092064

Upadhyay, A., Jaiswal, N., & Kuamr, A. (2025). Biofilm battle: New transformative tactics to tackle the bacterial biofilm infections. In Microbial Pathogenesis (Vol. 199). Academic Press. doi: 10.1016/j.micpath.2025.107277

Upadhyay, A., Pal, D., & Kumar, A. (2024). Development of the dynamic approach for detection of Salmonella Typhi biofilm via optimizations of interaction with safranin dye. *Biologia*. doi: 10.1007/s11756-024-01716-3

Van Roy, Z., & Kielian, T. (2025). Immune-based strategies for the treatment of biofilm infections. *Biofilm*, *9*. doi: 10.1016/j.bioflm.2025.100264

Varma, A., Warghane, A., Dhiman, N. K., Paserkar, N., Upadhye, V., Modi, A., & Saini, R. (2023). The role of nanocomposites against biofilm infections in humans. In Frontiers in Cellular and Infection Microbiology (Vol. 13). Frontiers Media S.A. doi: 10.3389/fcimb.2023.1104615

Veiko, A. G., Olchowik-Grabarek, E., Sekowski, S., Roszkowska, A., Lapshina, E. A., Dobrzynska, I., Zamaraeva, M., & Zavodnik, I. B. (2023). Antimicrobial Activity of Quercetin, Naringenin and Catechin: Flavonoids Inhibit Staphylococcus aureus-Induced Hemolysis and Modify Membranes of Bacteria and Erythrocytes. *Molecules*, *28*(3). doi: 10.3390/molecules28031252

Wi, Y., & Patel, R. (2018). Understanding Biofilms and Novel Approaches to the Diagnosis, Prevention, and Treatment of Medical Device-Associated Infections. *Infectious Disease Clinics of North America*, *32 4*, 915–929. doi: 10.1016/j.idc.2018.06.009

Wicaksono, W. A., Erschen, S., Krause, R., Müller, H., Cernava, T., & Berg, G. (2022). Enhanced survival of multi-species biofilms under stress is promoted by low-abundant but antimicrobial-resistant keystone species. *Journal of Hazardous Materials*, *422*, 126836. doi: 10.1016/J.JHAZMAT.2021.126836

Wong, L. L., Mugunthan, S., Kundukad, B., Ho, J., Rice, S., Hinks, J., Seviour, T., Parikh, A., & Kjelleberg, S. (2022). Microbial biofilms are shaped by the constant dialogue between biological and physical forces in the extracellular matrix. *Environmental Microbiology*. doi: 10.1111/1462-2920.16306

Xin, H., Chen, X., Shuyue, Chen, Z., Li, J., Fan, Q., Luo, H., Liu, G., & Li, Y. (2025). Stratification of bacterial communities and extracellular polymeric substances in anodic and cathodic biofilms of bioelectrochemical system under metronidazole stress in seawater. *Bioresource Technology*, 133040. doi: 10.1016/j.biortech.2025.133040

Xiu, W., Ren, L., Xiao, H., Zhang, Y., Wang, D., Yang, K., Wang, S., Yuwen, L., Li, X., Dong, H., Li, Q., Mou, Y., Zhang, Y., Yin, Z., Liang, B., Gao, Y., & Wang, L. (2023). *Ultrasound-responsive catalytic microbubbles enhance biofilm elimination and immune activation to treat chronic lung infections*.

Yi, L., Li, J., Liu, B., & Wang, Y. (2019). Advances in research on signal molecules regulating biofilms. In World Journal of Microbiology and Biotechnology (Vol. 35, Issue 8). Springer Netherlands. doi: 10.1007/s11274-019-2706-x

Yin, W., Wang, Y., Liu, L., & He, J. (2019). Biofilms: The Microbial "Protective Clothing" in Extreme Environments. *International Journal of Molecular Sciences*, *20*. doi: 10.3390/ijms20143423

Zhao, X., Yu, Z., & Ding, T. (2020). Quorum-sensing regulation of antimicrobial resistance in bacteria. In Microorganisms (Vol. 8, Issue 3). MDPI AG. doi: 10.3390/microorganisms8030425

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. In Frontiers in Microbiology (Vol. 11). Frontiers Media S.A. doi: 10.3389/fmicb.2020.589640

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