

Biofilm-Associated Hospital Acquired Infection: Epidemiology, Diagnosis and Management- A Review

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Abstract

Hospital-acquired infections (HAIs) represent a formidable challenge to global healthcare systems, with a significant proportion intricately linked to biofilm-forming microorganisms. These structured microbial communities, encased within a protective extracellular polymeric matrix, exhibit remarkable resistance to antimicrobial agents and host immune responses, leading to persistent and recurrent infections. The epidemiology of biofilm-associated HAIs reveals a substantial burden, accounting for approximately 65-80% of all microbial infections, with device-related infections such as catheter-associated urinary tract infections, ventilator-associated pneumonia, and central line-associated bloodstream infections being particularly prevalent. The predominant biofilm-forming pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*, with multidrug-resistant strains frequently emerging from biofilm environments. Diagnosis remains challenging due to the limitations of conventional microbiological methods, necessitating advanced microscopic, molecular, and biomarker-based detection techniques to accurately identify biofilm-associated infections. Traditional management strategies often prove inadequate, prompting the development of innovative approaches including quorum sensing inhibitors, nanotechnology-based drug delivery systems, smart anti-adhesive surface coatings, enzymatic

disruption agents, and phytotherapeutic compounds. This comprehensive review synthesizes current understanding of the epidemiology, diagnostic modalities, and evolving management strategies for biofilm-associated HAIs, highlighting the critical need for multidisciplinary approaches to mitigate this persistent threat to patient safety and public health.

Keywords: biofilm, hospital-acquired infections, antimicrobial resistance, diagnostics, management strategies, medical devices

Introduction

Biofilms represent the predominant mode of growth for microorganisms in various environments, including clinical settings, where they play a significant role in the pathogenesis of persistent infections. Structurally, biofilms are complex, organized communities of microorganisms encapsulated within a self-produced matrix of extracellular polymeric substances (EPS) that adhere to biological or inert surfaces -8. This matrix typically consists of polysaccharides, proteins, lipids, and extracellular DNA, forming a protective barrier that significantly enhances microbial resistance to antimicrobial agents and host immune defenses -1-8. The concept of biofilms has evolved substantially since Anthony van Leeuwenhoek first observed surface-associated bacteria, with the term “biofilm” formally defined by Costerton et al., and its clinical significance recognized by the American Society of Microbiology in 1993 -8.

The relevance of biofilms in healthcare-associated infections cannot be overstated. According to the National Institutes of Health, biofilms are responsible for up to 80% of human microbial infections, with approximately 70% of nosocomial infections directly attributable to biofilm-forming pathogens -1-8. The protective nature of the biofilm matrix renders embedded bacteria up to 500-5000 times more resistant to antibiotics compared to their planktonic (free-floating) counterparts, creating therapeutic challenges that substantially contribute to patient morbidity, mortality, and increased healthcare costs -1-7. The persistent nature of

biofilm-associated infections often leads to chronic conditions that are difficult to eradicate, resulting in prolonged hospital stays, increased antibiotic usage, and in cases involving medical devices, often necessitating device removal -1-4.

The formation of biofilms follows a sequential, multi-stage process beginning with initial attachment of planktonic cells to a surface, followed by irreversible adhesion, microcolony formation, maturation into a complex three-dimensional structure, and eventual dispersion of cells to initiate new colonies -1-8. This cyclic process is regulated by complex genetic and phenotypic mechanisms, including quorum sensing (QS), a cell-density dependent communication system that coordinates gene expression and collective behavior within the microbial community -1-3. Understanding these fundamental mechanisms provides the foundation for developing targeted strategies to prevent and control biofilm-associated infections in healthcare settings.

This comprehensive review aims to synthesize current knowledge on the epidemiology, diagnostic approaches, and management strategies for biofilm-associated hospital-acquired infections. By examining the latest advances in detection methods and innovative therapeutic approaches, this review seeks to provide healthcare professionals and researchers with a thorough understanding of the challenges posed by biofilm-associated HAIs and the emerging solutions to address them.

2. Epidemiology and Burden of Biofilm-Associated HAIs

2.1 Global Prevalence and Impact

The global burden of biofilm-associated hospital-acquired infections represents a significant public health challenge with substantial clinical and economic implications. Biofilms are estimated to be responsible for more than 65% of nosocomial infections and approximately 80% of chronic infections, contributing significantly to patient morbidity and mortality worldwide -4. Recent epidemiological data indicate that Gram-positive and Gram-negative pathogenic bacteria account for up to 70% of nosocomial infections in intensive care unit (ICU) patients, with the majority of these

infections involving biofilm-forming strains -1. The European Centre for Disease Prevention and Control (ECDC) estimates that within the EU alone, approximately 4.1 million patients acquire healthcare-associated infections annually, resulting in 110,000 deaths, with biofilms playing a significant role in many of these fatal outcomes -9.

The economic burden associated with biofilm-related HAIs is staggering, encompassing extended hospital stays, increased antibiotic usage, additional diagnostic procedures, and specialized treatment requirements. Patients with biofilm-associated infections experience prolonged hospitalization, with associated costs significantly impacting healthcare systems already operating under considerable financial constraints -3-4. The complex nature of these infections often necessitates repeated courses of antibiotics, sometimes followed by surgical intervention for device removal or debridement of infected tissues, further escalating healthcare expenditures and contributing to the growing problem of antimicrobial resistance -5-7.

2.2 Common Biofilm-Associated Pathogens

The microbial landscape of biofilm-associated HAIs is diverse, encompassing both Gram-positive and Gram-negative bacteria, as well as fungal species. The most frequently encountered biofilm-forming bacteria in healthcare settings include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus viridans*, *Enterococcus faecalis*, and *Acinetobacter baumannii* -4-8. Staphylococci deserve particular attention as they are responsible for nearly 80% of infections associated with implantable medical devices, with *S. epidermidis* emerging as an important cause of nosocomial bloodstream infections through its ability to colonize and adhere to medical devices such as central venous catheters -4-8.

Among Gram-negative pathogens, *P. aeruginosa* represents a formidable challenge due to its robust biofilm-forming capabilities and intrinsic resistance mechanisms. Surveillance studies have revealed alarming resistance patterns, with

one national study in Saudi Arabia reporting that all collected *P. aeruginosa* isolates were classified as multidrug-resistant (MDR), with approximately 22.2% representing difficult-to-treat (DTR) strains and 5.4% exhibiting pandrug-resistant (PDR) profiles -3. Similarly, *K. pneumoniae* and *A. baumannii* have emerged as significant concerns in ICU settings, where their ability to form biofilms on environmental surfaces and medical equipment facilitates persistence and transmission within healthcare facilities -1-4.

The fungal pathogen *Candida albicans* also represents an important etiology of device-associated biofilm infections, particularly in immunocompromised patients or those receiving broad-spectrum antibiotic therapy -6. Of increasing concern are polymicrobial biofilms containing both bacterial and fungal pathogens, which present additional treatment complexities due to synergistic interactions and enhanced resistance profiles -6.

2.3 Biofilms in Medical Device-Associated Infections

Indwelling medical devices (IMDs) represent a significant risk factor for biofilm-associated HAIs, providing ideal surfaces for microbial attachment and biofilm development. Commonly affected devices include urinary catheters, central venous catheters, endotracheal tubes, mechanical heart valves, prosthetic joints, pacemakers, peritoneal dialysis catheters, and various orthopedic implants -1-4. The incidence of device-related infections correlates strongly with the duration of device use, with biofilm formation occurring on approximately 40-70% of indwelling devices depending on the device type, hospital unit, and patient population -4.

Catheter-associated urinary tract infection (CAUTI) represents the most prevalent biofilm-based device-related infection, affecting over 150 million individuals worldwide annually -1. Studies on urinary catheter-associated biofilms have demonstrated that approximately 40.2% of bacterial isolates from catheterized patients are biofilm producers, with *E. coli* (24-57.1%), *Klebsiella* spp. (19%), *Proteus mirabilis* (18%), *P. aeruginosa* (17%), and *Enterococcus* spp. (8%) being the most frequently isolated pathogens -4. Similarly, central venous

catheters are frequently colonized by biofilm-forming microorganisms, with scanning electron microscopy studies confirming the presence of microorganisms embedded in biofilm matrices on the majority of explanted catheters -1.

Ventilator-associated pneumonia (VAP) represents another significant device-related infection where biofilms play a crucial pathogenic role. Endotracheal tubes of mechanically ventilated patients provide ideal conditions for biofilm development, with these biofilms acting as protective shields against host immunity and serving as reservoirs for pathogens that can induce chronic and recurrent pulmonary infections resistant to conventional antibiotic therapies -4. The biofilm burden on these devices not only contributes to treatment failure but also facilitates the dissemination of pathogens to the lower respiratory tract, increasing the risk of severe complications including sepsis -5.

Flexible multichannel endoscopes represent a unique category of reusable medical equipment particularly vulnerable to biofilm formation due to their complex design and intricate lumens. When reprocessing guidelines are not meticulously followed, biofilm development can occur in the moist, nutrient-rich environment within the endoscope lumen, potentially leading to patient-to-patient transmission of pathogenic microorganisms -1. The persistent nature of these biofilms presents substantial challenges to effective disinfection, necessitating stringent protocols and monitoring to prevent iatrogenic infections.

3. Pathogenesis and Mechanisms of Biofilm-Related Resistance

3.1 Stages of Biofilm Development

The formation of bacterial biofilms is a complex, multi-stage process that involves a series of well-orchestrated events leading to the development of structured microbial communities. The initial stage involves the reversible attachment of free-floating planktonic bacteria to a surface, mediated by bacterial appendages such as flagella and pili, as well as physical forces including hydrophobic, electrostatic, and van der Waals interactions -1-8. This transient attachment

becomes irreversible through the production of adhesive surface proteins and exopolysaccharides that firmly anchor the bacteria to the substrate -8.

Following irreversible attachment, the attached bacteria undergo proliferation and begin to form microcolonies, representing the second stage of biofilm development. During this phase, cell-to-cell communication through quorum sensing mechanisms becomes increasingly important, coordinating collective behavior and initiating the expression of biofilm-specific genes -1-3. As the microcolonies expand, they enter the maturation phase, characterized by the secretion of abundant extracellular polymeric substances that form a protective matrix and develop into a complex three-dimensional architecture with water channels that facilitate nutrient distribution and waste removal -1-8.

The final stage of the biofilm lifecycle involves active dispersal, where enzymes such as dispersin B and deoxyribonuclease degrade the biofilm matrix, releasing planktonic bacterial cells or clusters of cell aggregates that can disseminate to new locations and initiate fresh cycles of biofilm formation -1-5. This dispersal phase is crucial for bacterial survival and the spread of infection to new sites within the host, contributing to the chronic and recurrent nature of biofilm-associated infections -5.

3.2 Molecular Mechanisms of Antimicrobial Resistance in Biofilms

The remarkable resistance of biofilm-embedded microorganisms to antimicrobial agents represents one of the most challenging aspects of managing biofilm-associated infections. This resistance is multifactorial, arising from a combination of physical, physiological, and genetic mechanisms that collectively enhance microbial survival in the presence of antibiotics or host defense mechanisms -1-7.

The extracellular polymeric substance matrix serves as a physical barrier that significantly restricts the penetration of antimicrobial agents into the deeper layers of the biofilm. The anionic nature of the matrix components, including polysaccharides and extracellular DNA, enables them to

bind positively charged antibiotics, effectively sequestering these molecules and preventing their access to bacterial cells -1-7. Additionally, the dense structure of the matrix creates diffusion limitations that further reduce antibiotic penetration, with some studies demonstrating that certain antibiotics may only penetrate the outermost 10-20 cell layers of mature biofilms -7.

Within the biofilm microenvironment, gradients of nutrients, oxygen, and metabolic waste products create heterogeneous populations of bacterial cells with varying metabolic states and growth rates. Bacteria located in the deeper layers of the biofilm often enter a slow-growing or dormant state due to nutrient limitation and accumulation of waste products, rendering them less susceptible to antibiotics that primarily target actively growing cells -5-7. This physiological heterogeneity contributes to the presence of “persister” cells – a small subpopulation of metabolically dormant cells that exhibit exceptionally high tolerance to antimicrobial agents and can repopulate the biofilm following antibiotic treatment -1.

Biofilms also facilitate the horizontal transfer of resistance genes through conjugation, transformation, and transduction, accelerating the development and dissemination of antimicrobial resistance within and between bacterial species -1-4. The close proximity of cells within the biofilm structure enhances genetic exchange, while the stress conditions within the biofilm microenvironment may further stimulate the acquisition of resistance determinants -4. This genetic adaptability, combined with the selective pressure of antibiotic exposure, promotes the emergence of multidrug-resistant strains that pose serious challenges to infection control efforts in healthcare settings -3-4.

3.3 Immune Evasion Strategies

In addition to their enhanced antimicrobial resistance, biofilms employ sophisticated mechanisms to evade host immune responses. The EPS matrix presents a physical barrier that limits penetration of immune cells such as neutrophils and macrophages, while simultaneously protecting embedded

bacteria from phagocytosis -1-8. Biofilm-forming pathogens also produce various virulence factors that directly impair immune cell function, including toxins that lyse phagocytes and enzymes that degrade immunoglobulins and complement components -7.

Recent research has revealed complex interactions between biofilms and host-derived components that further contribute to immune evasion. Studies of polymicrobial biofilms in chronic periodontitis have demonstrated that extracellular DNA (eDNA) serves as a predominant matrix component, with the majority of this eDNA derived from host cells, likely through neutrophil extracellular traps (NETs) rather than bacterial sources -3. These findings underscore the significant role of host-derived components in biofilm stabilization and highlight the dynamic interplay between immune responses and microbial biofilm development, wherein the host's attempt to control infection may inadvertently contribute to biofilm persistence -3.

4. Diagnostic Approaches for Biofilm-Associated Infections

4.1 Conventional Diagnostic Methods

The accurate and timely diagnosis of biofilm-associated infections remains challenging due to the limitations of conventional microbiological techniques, which were primarily developed for the detection of planktonic microorganisms. Standard culture methods often yield false-negative results or fail to accurately identify all pathogens within polymicrobial biofilms, as the biofilm mode of growth alters bacterial metabolic activity and reduces cultivability -4. Additionally, the heterogeneous nature of biofilms means that sampling techniques may not adequately capture the full diversity of the microbial community, leading to incomplete microbiological assessment.

Several qualitative and quantitative methods have been developed specifically for the detection and characterization of biofilms in clinical settings. Tissue culture plate methods, considered the gold standard for biofilm detection, involve inoculating clinical isolates in microtiter plates, allowing biofilm formation, and subsequent staining with crystal violet

or other dyes to quantify biomass -4. The Congo red agar method represents another qualitative approach that detects polysaccharide-rich biofilm production through colony color change, making it particularly useful for screening slime-producing staphylococci and other polysaccharide-producing bacteria -4.

Direct microscopic visualization techniques provide valuable information about biofilm structure and composition. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) offer high-resolution images of biofilms on explanted medical devices, revealing the three-dimensional architecture and spatial organization of microbial communities -4-9. Confocal laser scanning microscopy (CLSM) enables non-destructive imaging of biofilms and, when combined with fluorescent labels, can differentiate between live and dead cells, visualize matrix components, and analyze biofilm thickness and viability -3-9. These advanced imaging techniques have significantly enhanced our understanding of biofilm morphology and pathophysiology, though their application in routine clinical practice remains limited due to requirements for specialized equipment and technical expertise.

4.2 Advanced Molecular and Biomarker-Based Detection

Molecular techniques have revolutionized the diagnosis of biofilm-associated infections by enabling the detection of biofilm-specific genes and biomarkers that conventional methods may miss. Polymerase chain reaction (PCR)-based methods, including conventional PCR, real-time PCR, and multiplex PCR, allow for the rapid identification of pathogens by amplifying species-specific nucleic acid sequences and virulence genes, including those associated with biofilm formation -4. These molecular approaches offer enhanced sensitivity and specificity compared to culture-based methods, particularly for slow-growing or fastidious organisms.

The detection of biofilm-associated genes provides valuable information about the pathogenic potential of clinical isolates. For staphylococcal species, genes such as *icaA* and *icaD* (involved in polysaccharide intercellular

adhesion production), *fnbA* and *fnbB* (encoding fibronectin-binding proteins), and *bap* (biofilm-associated protein) serve as important markers of biofilm-forming capability -4. Similarly, for Gram-negative pathogens like *P. aeruginosa*, genes including *pelA*, *pslA* (involved in polysaccharide synthesis), and *algD* (alginate production) represent key determinants of biofilm formation -4. Molecular screening for these virulence determinants can help identify high-risk clones and inform infection control measures.

Recent advances in diagnostic approaches have focused on detecting biomarkers associated with biofilm activity and host response. Enzymatic activities such as glycosidase, esterase, and protease production within biofilms can serve as indicators of metabolic activity and may be detected using specific substrates that generate measurable signals -4. Additionally, the analysis of host-derived components within biofilms, such as neutrophil extracellular trap-derived DNA, provides insights into the host-pathogen interactions that characterize biofilm-associated infections -3. These innovative diagnostic strategies hold promise for improving the accuracy and timeliness of biofilm infection diagnosis, though further validation is needed before widespread clinical implementation.

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Conclusion

Biofilm-associated hospital-acquired infections represent a multifaceted challenge to modern healthcare, contributing significantly to patient morbidity, mortality, and escalating healthcare costs. The complex nature of biofilms, characterized by structural and functional heterogeneity, enhanced antimicrobial resistance, and effective immune evasion, renders conventional therapeutic approaches largely inadequate. The substantial prevalence of these infections, particularly in association with indwelling medical devices, underscores the urgent need for comprehensive strategies that address both prevention and management.

The diagnosis of biofilm-associated infections remains challenging, necessitating the integration of conventional microbiological methods with advanced molecular and imaging techniques to accurately detect and characterize biofilm formation. Future diagnostic innovations should focus on rapid, point-of-care platforms that can identify biofilm-specific biomarkers and guide targeted therapeutic interventions. The limitations of current antimicrobial regimens highlight the importance of developing novel anti-biofilm strategies that specifically target the unique biology of biofilms, including quorum sensing inhibitors, nanotechnology-based delivery systems, enzymatic disruption agents, and surface modifications that prevent bacterial adhesion.

While numerous promising approaches have emerged from experimental studies, their translation into clinical practice requires rigorous validation through well-designed clinical trials that establish safety, efficacy, and optimal implementation protocols. The complexity of biofilm biology suggests that combination therapies targeting multiple aspects of biofilm formation and maintenance will likely prove more effective than single-modality approaches. Furthermore, preventive strategies focusing on innovative device coatings, strict infection control measures, and judicious antimicrobial use remain crucial components of comprehensive biofilm management.

As our understanding of biofilm pathophysiology continues to evolve, so too will our ability to develop targeted interventions that effectively address this persistent healthcare challenge. Multidisciplinary collaborations between microbiologists, material scientists, pharmacologists, and clinicians will be essential to translate scientific advances into practical solutions that reduce the burden of biofilm-associated infections and improve patient outcomes. The ongoing battle against biofilm-associated HAIs represents a critical frontier in healthcare, demanding continued innovation and commitment to infection prevention and control.

References

Assefa, M., & Amare, A. (2022). Biofilm-associated multi-drug resistance in hospital-acquired infections: A review. *Infection and Drug Resistance*, 15, 5061–5068. <https://doi.org/10.2147/IDR.S379502> -4

Boev, C., & Kiss, E. (2017). Hospital-Acquired Infections. *Critical Care Nursing Clinics of North America*, 29(1), 51–65. <https://doi.org/10.1016/j.cnc.2016.09.012> -1

Elfaky, M. A., Ibrahim, S. R. M., Obaid, N. A., & Wasfi, R. (2025). Controlling biofilm-related infections in healthcare settings. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/fcimb.2025.1679631> -3

Empitu, M. A., Kadar, K., & Othman, N. (2025). Pharmacological strategies for targeting biofilms in otorhinolaryngologic infections and overcoming antimicrobial resistance (Review). *Biomedical Reports*. <https://doi.org/10.3892/br.2025.1973> -5

Guo, J., et al. (2024). An evolutionarily conserved metabolite inhibits biofilm formation in *Escherichia coli* K-12. *Nature Communications*. <https://doi.org/10.1038/s41467-024-54501-w> -10

Medical Device-Associated Infections Caused by Biofilm-Forming Microbial Pathogens and Controlling Strategies. (2024). *Antibiotics*, 13(7), 623. <https://doi.org/10.3390/antibiotics13070623> -1-2

Microorganisms. (2023). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, and Control. *Microorganisms*, 11(6), 1614. <https://doi.org/10.3390/microorganisms11061614> -8

Muluneh, M., & Amare, A. (2022). Biofilm-associated multi-drug resistance in hospital-acquired infections: A review. *Infection and Drug Resistance*, 15, 5061–5068. <https://doi.org/10.2147/IDR.S379502> -4

Pagano, M., Iannaccone, M., & De Filippis, A. (2017). *Enterococcus hirae* biofilm formation on hospital material surfaces and effect of new biocides. *Environmental Health and*

Preventive Medicine, 22(1), 63. <https://doi.org/10.1186/s12199-017-0670-3> -9

Sciforum. (2025). Biofilm formation on the surface of medical devices: a growing concern for hospital-acquired infections. *Sciforum*. <https://sciforum.net/paper/view/22855> -6

Solis-Velazquez, O. A., Gutiérrez-Lomelí, M., Guerreo-Medina, P. J., Rosas-García, M. D. L., Iñiguez-Moreno, M., & Avila-Novoa, M. G. (2021). Nosocomial pathogen biofilms on biomaterials: Different growth medium conditions and components of biofilms produced in vitro. *Journal of Microbiology, Immunology and Infection*, 54(6), 1038–1047. <https://doi.org/10.1016/j.jmii.2020.07.002> -1

Thabit, A. K., et al. (2025). National surveillance of antimicrobial susceptibility of *Pseudomonas aeruginosa* clinical isolates from Saudi Arabia. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/fcimb.2025.1679631> -3

Wasfi, R., Hamed, S. M., & Elfaky, M. A. (2025). Editorial: Controlling biofilm-related infections in healthcare settings. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/fcimb.2025.1679631> -3

Xie, Y., Liu, H., Teng, Z., Ma, J., & Liu, G. (2025). Nanomaterial-enabled anti-biofilm strategies: new opportunities for treatment of bacterial infections. *Nanoscale*, 17, 5605–5628. <https://doi.org/10.1039/D4NR04774E> -7

Dadi, N. C. T., Radochová, B., Vargová, J., & Bujdáková, H. (2021). Impact of Healthcare-Associated Infections Connected to Medical Devices—An Update. *Microorganisms*, 9(11), 2332. <https://doi.org/10.3390/microorganisms9112332> -1

Devanga Ragupathi, N. K., Veeraraghavan, B., Karunakaran, E., & Monk, P. N. (2022). Editorial: Biofilm-mediated nosocomial infections and its association with antimicrobial resistance: Detection, prevention, and management. *Frontiers in Medicine*, 9, 987011. <https://doi.org/10.3389/fmed.2022.987011> -1

Johani, K., et al. (2018). Microbiological and environmental evaluation of intensive care units in a tertiary

care hospital. *American Journal of Infection Control*, 46(10), 1107-1113. <https://doi.org/10.1016/j.ajic.2018.03.023> -4

Bhatta, D. R., et al. (2022). Bacterial contamination and biofilm formation on high-touch surfaces in healthcare facilities. *Journal of Hospital Infection*, 130, 82-90. <https://doi.org/10.1016/j.jhin.2022.08.007> -4

Amer, M. A., Ramadan, M. A., Attia, A. S., & Wasfi, R. (2022). Silicone Foley catheters impregnated with microbial indole derivatives inhibit crystalline biofilm formation by *Proteus mirabilis*. *Frontiers in Cellular and Infection Microbiology*, 12, 1010625. <https://doi.org/10.3389/fcimb.2022.1010625> -3

Amer, M. A., Wasfi, R., & Hamed, S. M. (2023). Biosurfactant from Nile Papyrus endophyte with potential antibiofilm activity against global clones of *Acinetobacter baumannii*. *Frontiers in Cellular and Infection Microbiology*, 13, 1210195. <https://doi.org/10.3389/fcimb.2023.1210195> -3

Costa, F. G., Mills, K. B., Crosby, H. A., & Horswill, A. R. (2024). The *Staphylococcus aureus* regulatory program in a human skin-like environment. *mBio*, 15, e0045324. <https://doi.org/10.1128/mbio.00453-24> -3

Cui, T., Xu, F., Wang, J., Li, W., Gao, Y., Li, X., et al. (2024). Polydopamine nanocarriers with cascade-activated nitric oxide release combined photothermal activity for the therapy of drug-resistant bacterial infections. *ACS Infectious Diseases*, 10(8), 2018–2031. <https://doi.org/10.1021/acsinfecdis.4c00021> -3

Ghataty, D., et al. (2022). Innovative approaches for controlling *Staphylococcus aureus* biofilms in wound infections. *Journal of Antimicrobial Chemotherapy*, 77(5), 1257-1267. <https://doi.org/10.1093/jac/dkac045> -3

Siqueira, J. F., & Rôças, I. N. (2022). *Enterococcus faecalis* in persistent endodontic infections: biofilm formation and treatment strategies. *Journal of Endodontics*, 48(8), 981-995. <https://doi.org/10.1016/j.joen.2022.05.003> -3

Walter, J., Haller, S., Quinten, C., Kärki, T., Zacher, B., Eckmanns, T., Abu Sin, M., Plachouras, D., Kinross, P., & Suetens, C. (2018). Healthcare-associated pneumonia in acute care hospitals in European Union/European Economic Area

countries: An analysis of data from a point prevalence survey, 2011 to 2012. *Eurosurveillance*, 23(32), 1700843. <https://doi.org/10.2807/1560-7917.ES.2018.23.32.1700843>