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Review Article

Biofilm-associated healthcare-associated infections: mechanisms, clinical burden, and emerging therapeutic frontiers – a narrative review

Biofilm-assoziierte nosokomiale Infektionen: Mechanismen, klinische Belastung und therapeutische Herausforderungen – ein narratives Review

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Abstract

Biofilms are highly organized microbial communities embedded within an extracellular polymeric matrix and represent a critical yet underappreciated driver of healthcare-associated infections (HAIs). Their ability to colonize medical devices and host tissues underlies major infections, including central line-associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonia, and surgical site infections.

Biofilm formation is dynamic, multistage process involving attachment, maturation, and dispersal of microorganisms, each phase reinforcing persistence and therapeutic failure. The matrix impedes immune clearance and antimicrobial penetration, while quorum sensing, metabolic heterogeneity, and persister cell formation further enhance tolerance. Clinically important bacterial and fungal biofilms, particularly those formed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Candida species, exhibit resistance far exceeding planktonic cells. Diagnostic limitations obscure true disease burden, and current strategies relying on antimicrobials and device removal remain inadequate amid rising resistance.

This manuscript provides comprehensive synthesis of existing and novel strategies to mitigate HAIs, with particular emphasis on translational relevance.

Keywords: antimicrobial stewardship, central venous catheter, urinary catheters, endotracheal tubes, prosthetic implants, mortality

Zusammenfassung

Biofilme sind hochorganisierte mikrobielle Gemeinschaften, die in eine extrazelluläre Polymermatrix eingebettet sind und einen kritischen, aber unterschätzten Auslöser nosokomialer Infektionen (NI) darstellen. Ihre Fähigkeit, Devices und Wirtsgewebe zu besiedeln, ist Ursache für schwere Infektionen, darunter ZVK-assoziierte Blutstrominfektionen, Katheter-assoziierte Harnwegsinfektionen, Beatmungs-assoziierte Pneumonien und postoperative Wundinfektionen.

Die Bildung von Biofilmen ist ein dynamischer, mehrstufiger Prozess, der Anlagerung, Reifung und Ausbreitung von Mikroorganismen umfasst, wobei jede Phase die Persistenz und das Therapieversagen verstärkt. Die Matrix behindert die Immunabwehr und die antimikrobielle Penetration, während Quorum Sensing, metabolische Heterogenität und Persistenzzellbildung die Toleranz erhöhen. Klinisch wichtige bakterielle und pilzliche Biofilme, insbesondere solche, die von *Staphylococcus aureus*, *Pseudomonas aeruginosa* und Candida-Arten gebildet werden, weisen eine Resistenz auf, die weit über die von Planktonzellen hinausgeht. Diagnostische Einschränkungen verschleiern die tatsächliche Krank-

heitslast, und aktuelle Strategien, die auf antimikrobiellen Mitteln und der Entfernung von Devices basieren, bleiben angesichts zunehmender Resistenzen unzureichend.
Das Manuskript bietet eine Zusammenführung bestehender und neuer Strategien zur Reduzierung von NI mit besonderem Schwerpunkt auf der translationalen Relevanz.

Schlüsselwörter: Antibiotic Stewardship, zentraler Venenkatheter, Harnwegkatheter, Endotrachealtuben, Gelenkimplantationen, Mortalität

Introduction

Healthcare-associated infections (HAIs) remain among the most frequent and devastating complications in modern medicine, representing a major global public health challenge [1]. Microbial biofilms underlie the majority of device-associated infections, are implicated in up to 70% of all microbial diseases and represent a major driver of HAIs [2]. Once established on indwelling medical devices such as central venous catheters (CVCs), urinary catheters, endotracheal tubes (ET), and prosthetic implants, biofilms form a highly resistant microbial reservoir that is nearly impossible to eradicate with standard antibiotic regimens [3], [4], [5], [6], [7]. This persistent mode of infection not only complicates clinical management but also accelerates the development and dissemination of antimicrobial resistance (AMR), with an estimated annual economic burden exceeding USD 5 trillion [8], [9].
With the increasing use of invasive medical devices in contemporary healthcare, the incidence of biofilm-associated infections has escalated globally [10]. Virtually all implanted or indwelling devices are susceptible to microbial colonization, turning life-saving interventions into potential sources of chronic infection [4]. Biofilm-associated infections now account for the majority of persistent bacterial infections in humans and are implicated in nearly half of all HAIs linked to medical devices [11].
Understanding biofilms is essential to tackling HAIs, as nearly 80% of chronic infections are biofilm-associated [12]. Their inherent resistance to antibiotics fuels persistent infections and the rise of multidrug-resistant pathogens, projected to cause 10 million deaths annually by 2050 [13]. However, current infection control and treatment strategies, largely designed for planktonic bacteria, fail to adequately address biofilm-associated infections, particularly on medical devices and sterile sites [14], [15], [16]. The interplay between HAIs and biofilms represents a silent yet escalating threat to patient safety and healthcare systems worldwide. This review seeks to close the critical gap between microbiological insight and clinical practice by translating the complex biology of biofilms into actionable strategies for the prevention, diagnosis, and treatment of HAIs. Addressing this challenge will require not only improved infection prevention protocols and device stewardship but also urgent innovation in anti-biofilm strategies and antimicrobial development.

Method

A narrative review was conducted to evaluate the role of biofilms in HAIs, AMR, and emerging anti-biofilm strategies. Relevant articles were identified through search of PubMed using keywords such as “biofilm”, “healthcare-associated infections”, “device-associated infections”, “antimicrobial resistance”, “quorum sensing”, and “anti-biofilm therapy”. Original research articles, reviews, clinical studies, and guideline documents published in English were included. The retrieved literature was screened for relevance, and eligible studies were critically reviewed and synthesized to provide an overview of biofilm biology, clinical significance, diagnostic challenges, and current and emerging management approaches.

Results

The biofilm life cycle: a dynamic survival strategy of pathogens

Biofilms represent a unifying pathogenic strategy across bacterial and fungal pathogens implicated in HAIs [10]. They enable microbial persistence on indwelling medical devices, hospital surfaces, and host tissues, thereby driving chronicity, therapeutic failure, and recurrence [17]. Unlike planktonic organisms, biofilm-embedded pathogens display recalcitrance, a coordinated, multicellular phenotype of tolerance to antibiotics even at high concentrations and immune evasion that is central to the epidemiology of ventilator-associated pneumonia (VAP), catheter-associated urinary tract infection (CAUTI), central line-associated bloodstream infection (CLABSI) and periprosthetic joint infections (PJI) [18].

Formation and maturation of bacterial biofilm

Bacterial biofilm development (Figure 1) follows a stepwise progression:

- Initial attachment (reversible docking): Microbes transiently adhere to host or device surfaces through weak physicochemical interactions – hydrophobic forces, van der Waals attractions, and electrostatic charges, guided by environmental cues and bacterial motility [19].

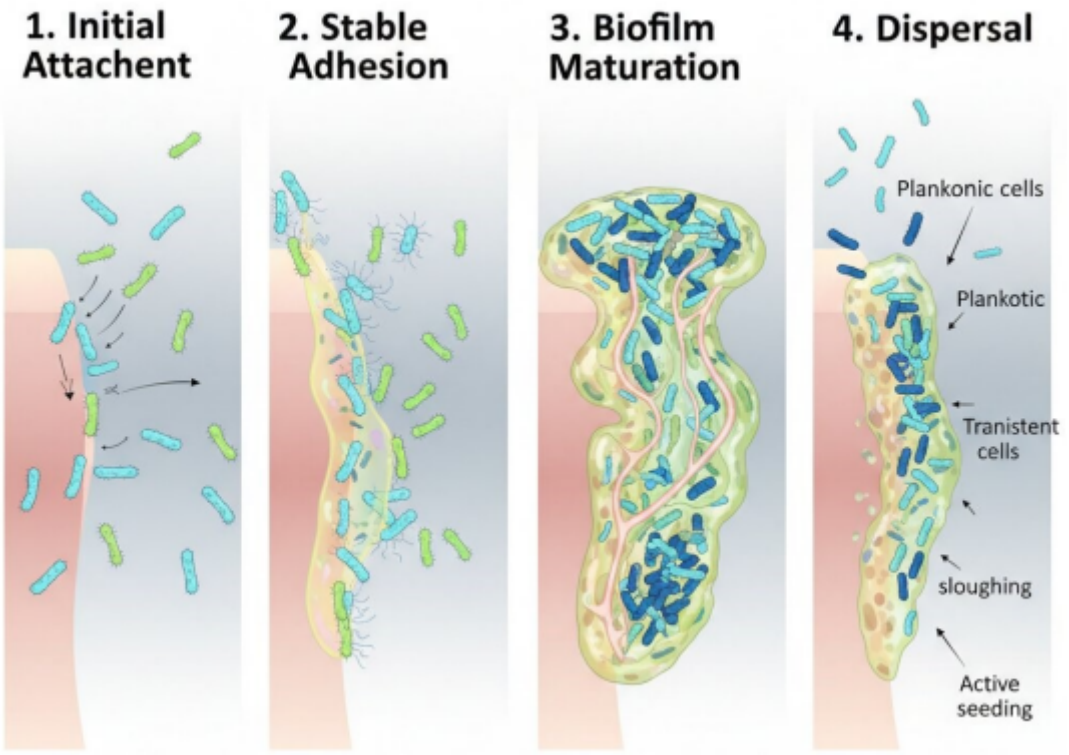

Figure 1: Formation and maturation of bacterial biofilm

- Stable adhesion (irreversible locking): Bacterial surface structures such as pili, fimbriae, and adhesins anchor the cells firmly. This molecular "handshake" triggers the secretion of extracellular polymeric substances (EPS), marking the transition from a free-living to a sessile lifestyle [19].
- Biofilm maturation (structural and functional expansion): Rapid bacterial replication, EPS accumulation, and spatial organization form complex three-dimensional communities with nutrient channels and protective architecture. These mature biofilms resist immune clearance and antimicrobial penetration [19].
- Dispersal (seeding of new niches): Environmental and quorum-sensing (QS) signals activate biofilm dispersal, releasing planktonic cells to colonize new surfaces. Dispersal may occur via erosion (gradual shedding), sloughing (bulk release), or active seeding (from within) [19].

Quorum sensing – the molecular orchestration of bacterial biofilms

Biofilm formation is not a random bacterial aggregation but a highly regulated, multicellular behaviour, coordinated through QS, a chemical communication system that enables bacteria to sense and respond to population density [20]. As bacterial numbers rise, diffusible signaling molecules, such as N-acyl homoserine lactones in Gram-negative species, autoinducing peptides in Gram-positives, and the interspecies autoinducer-2, accumulate in the extracellular environment [20]. Once a critical threshold is reached, these molecules trigger synchronized gene expression across the community. QS regulates both biofilm maturation and dispersal, facilitating colonization of new niches [20]. In clinical settings, this network contributes to persistent device-associated infections (DAI) and therapeutic failure. However, most mechanistic insights into QS are derived from controlled in vitro systems, and the complexity of polymicrobial interactions along with host immune factors in vivo may significantly alter QS dynamics, thereby limiting the translational applicability of these findings to real-world infection settings [21].

The extracellular matrix (ECM) – architect and shield of the bacterial biofilm

EPS is the biochemical framework that transforms bacterial aggregates into resilient biofilms. Composed of polysaccharides, proteins, lipids, and extracellular DNA, it anchors cells, maintains structural integrity, and protects against environmental stress [22]. EPS facilitates nutrient retention, immune evasion, and antibiotic tolerance by limiting drug penetration and host defenses [22]. It also promotes QS and horizontal gene transfer, enhancing antimicrobial resistance. Thus, EPS functions as both the structural scaffold and protective barrier underlying biofilm persistence [22]. Nevertheless, the relative contribution of EPS-mediated resistance versus metabolic dormancy in clinical infections remains debated, as antibiotics can penetrate biofilms yet fail due to physiological heterogeneity [23], [24].

Candida biofilms: a parallel fungal survival strategy

Fungal pathogens, particularly Candida species, mirror bacterial biofilm strategies but with following unique features (Figure 2) relevant to HAIs:

- Initial adhesion: Yeast cells rapidly adhere to biotic or abiotic surfaces, including mucosal epithelia and medical implants. This initial contact is mediated by adhesins, cell wall mannoproteins, and electrostatic interactions [25].
- Early biofilm development: Adherent yeast cells proliferate to form a basal monolayer. Hyphal transformation begins, a critical virulence trait, accompanied by the secretion of an ECM, rich in β-glucans, proteins, and extracellular DNA [25].
- Maturation: The biofilm architecture becomes increasingly complex, with dense networks of yeast, hyphae, and pseudo hyphae encased in ECM. Water channels form within the matrix, facilitating nutrient flow and metabolic cooperation. Antifungal resistance escalates sharply at this stage [25].
- Dispersal: Mature biofilms release yeast cells or hyphal fragments into the bloodstream or surrounding tissues. These disseminated cells exhibit an enhanced capacity to colonize new niches, contributing to recurrent and invasive candidiasis [25].

Quorum sensing in Candida – a driver of biofilm resilience

In *Candida (C.) albicans*, QS orchestrates biofilm development and drug resistance through population-dependent signaling [26]. The QS molecule farnesol inhibits hyphal formation by repressing hypha-associated genes (TUP1, CRK1) and upregulating efflux transporters (CDR1, PDR16), tipping the balance toward a biofilm-embedded phenotype [26]. Histidine kinase CHK1 mediates farnesol responsiveness, with CHK1 mutants forming biofilms despite QS inhibition [26]. Farnesol also contributes to biofilm dispersal under nutrient stress, enabling colonization of new sites. QS may regulate surface adhesion via anti-adhesins like YWP1, dampening biofilm adherence in response to population cues [26].

Extracellular matrix – the structural and protective backbone of Candida biofilms

In *C. albicans*, the ECM forms a dense, polymeric shield critical to biofilm architecture and defense. Composed of proteins, chitin, extracellular DNA, and β-1,3-glucans, this matrix stabilizes the biofilm's complex 3D structure, spanning 50 to 350 µm, and anchors cells against shear forces and antifungal insults [26]. Beyond physical integrity, the ECM serves as a chemical fortress, impeding immune cell access and sequestering antifungal drugs, thus enabling persistent infection [26].

Molecular basis of biofilm-mediated resistance

Three interdependent mechanisms form the backbone of biofilm resilience [27]:

- Physical barrier – the biofilm matrix impairs antibiotic penetration.
- Microenvironmental protection – nutrient gradients, acidic pH, hypoxia, and waste accumulation suppress antibiotic efficacy.
- Persistence – dormant subpopulations ("persister cells") evade killing due to metabolic inactivity.

This resistance is further amplified by biochemical defences (extracellular polysaccharides, eDNA, antibiotic-degrading enzymes, efflux pumps), molecular adaptations (horizontal gene transfer, mutational plasticity), and host–pathogen interactions (sub-inhibitory drug exposure, oxidative stress, QS) [27]. Collectively, these layered defences drive chronic infection and therapeutic failure in HAI.

Why conventional antibiotics fail against biofilms

Despite being genetically identical, bacteria within biofilms exhibit markedly reduced susceptibility to antibiotics compared to planktonic cells. This is not primarily due to classical resistance mechanisms but results from the structural, physiological, and metabolic heterogeneity of the biofilm state [19].

The EPS matrix acts as a diffusion barrier, limiting antibiotic penetration, particularly for aminoglycosides. However, incomplete efficacy cannot be explained by penetration alone [19]. Microenvironmental gradients of oxygen, nutrients, and pH create metabolically inactive zones, where bacteria become intrinsically tolerant to antibiotics targeting active cellular processes. In addition, biofilms contain persister cells – dormant, non-replicative variants that survive antibiotic exposure without genetic resistance and regain susceptibility upon regrowth [19].

However, most of these mechanisms have been predominantly demonstrated in *in vitro* models, and their relative contribution in complex *in vivo* biofilm-associated infections remains incompletely understood [28], [29]. In clinical settings, this multifactorial tolerance contributes to persistent DAI, often necessitating device removal and prolonged combination antimicrobial therapy, thereby increasing healthcare costs and patient morbidity [30], [31].

Why conventional antifungals fail against biofilms

Fungal biofilms, particularly those of Candida species, show markedly increased resistance to antifungals compared to planktonic cells [26]. This is due to a protective extracellular matrix, metabolic dormancy, and the pres-

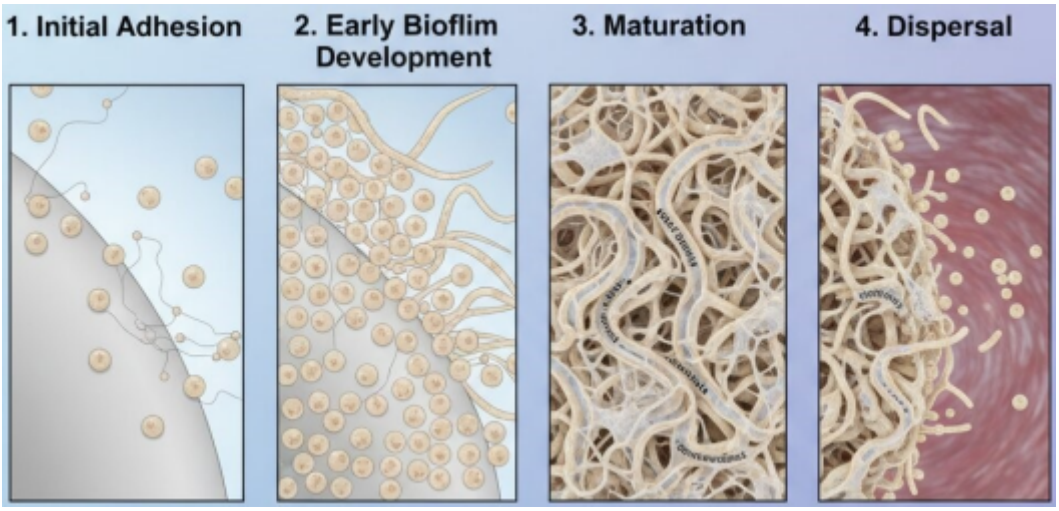

Figure 2: Formation and maturation of fungal biofilm

ence of persister cells. QS and high cell density further alter antifungal susceptibility [26]. These adaptive, non-genetic mechanisms render conventional therapies largely ineffective. In clinical settings, Candida biofilms frequently exist as part of polymicrobial communities, particularly with *Staphylococcus (S.) aureus*, where cross-kingdom interactions enhance biofilm robustness, antifungal tolerance, and virulence [32], [33].

Mechanisms of tolerance and "persister" cells

Biofilm-associated antibiotic tolerance arises predominantly from non-genetic, physiological adaptations rather than conventional resistance mechanisms. The biofilm's ECM limits antimicrobial penetration, while nutrient gradients and microenvironmental stressors create zones of low metabolic activity that reduce antibiotic efficacy [22], [34], [35]. Additionally, changes in cellular physiology, such as slowed growth, altered redox states, and stress response activation, further impair antibiotic action. These features collectively confer a reversible, phenotypic tolerance that resolves upon biofilm dispersion. In addition to these innate defenses, a critical subpopulation of cells, termed persisters, enter a dormant, non-replicative state that renders them transiently tolerant to antibiotics [22], [34], [35]. Unlike genetically resistant strains, persister cells do not harbor resistance mutations and regain susceptibility upon regrowth. However, their survival under antimicrobial pressure enables recurrence and may facilitate the eventual emergence of resistant mutants [22], [34], [35]. Together, these mechanisms make biofilm infections uniquely refractory to treatment.

Clinically relevant biofilm-forming pathogens

Most commonly associated biofilm-forming microorganisms linked with device-associated infections are *S. aureus, S. epidermidis, S. capitis, S. lugdunensis, Enterococcus (E.) faecalis, E. coli, Klebsiella (K.) pneumoniae, Enterobacter* spp., *Proteus (P.) mirabilis, Pseudomonas (P.) aeruginosa, Candida (C.) auris, C. albicans* and *C. rugosa* [19], [36], [37], [38], [39], [40].

Where biofilms thrive – clinical hotspots and devices

An estimated 65% of microbial infections and up to 80% of chronic infections are linked to biofilm formation. These highly structured microbial communities colonize both host tissues and indwelling medical devices, including prosthetic implants, CVCs, urinary catheters, and ET – as well as hospital environmental reservoirs such as sinks, tubing, and surfaces [41]. In clinical environments, biofilms act as persistent reservoirs for nosocomial pathogens, driving AMR and fuelling recurrent infections despite targeted therapy [42].

Common device-associated niches

Medical devices serve as prime substrates for biofilm development, transforming life-saving interventions into persistent infection reservoirs. Biofilm-associated infections are central to the burden of CLABSI, CAUTI, VAP and SSI, particularly those linked to prosthetic implants.

CVCs are major sites of biofilm formation and account for a significant proportion of CLABSI [43]. Biofilms form rapidly on catheter surfaces, with short-term use (<10 days) associated with extraluminal colonization and long-term use (>30 days) favouring intraluminal biofilm development [44]. Common pathogens include *S. aureus, S. epidermidis, E. faecalis, K. pneumoniae, P. aeruginosa*, and *C. albicans* [38]. These biofilms evade host immunity and antimicrobial therapy, often leading to septicemia and complications such as endocarditis, making prevention through aseptic techniques and antimicrobial-impregnated catheters essential [45].

The ET play a central role in VAP by impairing mucociliary clearance, suppressing cough reflexes, and facilitating entry of oropharyngeal flora into the lower airways [46], [47]. Biofilms forming on the internal lumen act as persistent reservoirs that protect pathogens from antibiotics

and host defenses, contributing to relapse and prolonged infection [48], [49]. VAP is predominantly caused by highly virulent and multidrug-resistant ESKAPE pathogens: *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* spp. – with *E. coli* increasingly implicated [50]. Despite advances in infection control practices, complete eradication of device-associated biofilms remains challenging, and clinical management often relies on device removal rather than pharmacological eradication alone [51].

Urinary catheters are a key risk factor for CAUTI, with most long-term catheterized patients developing bacteriuria [52]. Biofilms rapidly form on catheter surfaces, facilitating ascending infection by pathogens such as *E. coli, P.s mirabilis, P. aeruginosa, E. faecalis*, and *S. epidermidis*, often in polymicrobial communities [53]. These biofilms can obstruct urinary flow, induce inflammation, and predispose to urosepsis, highlighting the importance of minimizing catheter duration, maintaining closed systems, and using antimicrobial-coated devices [52].

Orthopaedic implants are highly prone to biofilm-associated SSIs, which may present as acute, chronic, or hematogenous infections [54]. Biofilm-embedded pathogens, including *S. aureus* (including MRSA), *S. epidermidis, E. faecalis, P. aeruginosa*, and *K. pneumoniae*, form polymicrobial consortia that resist host defences and antibiotic therapy [54], [55]. Persistent infection leads to chronic inflammation, osteolysis, implant loosening, and prosthetic failure, necessitating preventive strategies including perioperative prophylaxis, strict asepsis, and development of anti-biofilm implant surfaces [41], [56], [57].

Device surface determinants of biofilm formation

Surface roughness, material composition, and interfacial physicochemical properties are not passive features but key drivers of microbial adhesion and biofilm maturation. Increased surface roughness promotes microbial retention by expanding surface area and creating protective niches that reduce shear-mediated detachment, leading to greater early biofilm accumulation on rougher implant surfaces [58]. Beyond topography, material composition and surface chemistry, such as surface free energy, hydrophobicity, and charge, govern the strength and specificity of bacterial attachment, with hydrophobic interactions often favoring pathogen adherence [59]. Additionally, differences in biomaterials (e.g., titanium, zirconia, polymers) and their surface modifications significantly influence biofilm burden, highlighting the absence of a universally biofilm-resistant material [60]. Following implantation, rapid conditioning by host proteins like fibrinogen and fibronectin further enhances microbial colonization [59]. Collectively, these factors highlight how device-related properties shape microbial colonization, directly defining where biofilms thrive.

Biofilms as hidden drivers of healthcare-associated infections

Diagnostic blind spots – why biofilms escape detection

Despite their critical role in DAI, biofilms remain diagnostically elusive due to fundamental limitations in current detection methods as shown in Table 1 [61]. Standard cultures may yield false negatives due to low metabolic activity and poor recovery of biofilm-embedded organisms; moreover, even when positive, they detect only planktonic cells and fail to identify the sessile biofilm phenotype, including EPS matrix and altered physiology [61], [62], [63]. Routine imaging similarly lacks sensitivity for detecting biofilms on indwelling devices such as catheters, prostheses, and ETs. These limitations contribute to underrecognition of biofilm-mediated infections and may explain the discordance between in vitro susceptibility results and clinical outcomes [61], [62], [63]. Although standardized in vitro models (e.g., American Society for Testing and Materials) exist, they fail to replicate the dynamic and heterogeneous nature of in vivo biofilms, and laboratory strains may lose biofilm-forming capacity over time [64]. Emerging approaches, including experimental biomarkers and artificial intelligence, show promise for early non-invasive detection; however, the lack of validated biofilm-specific biomarkers remains a major limitation [65], [66], [67], [68], [69], [70], [71], [72]. While advanced molecular and imaging techniques – such as 16S rRNA sequencing, next-generation sequencing, sonication, Confocal Microscopy, cryo-SEM, and atomic force microscopy – provide improved insights, their limited scalability restricts routine clinical use, leaving a persistent gap in the early and accurate diagnosis of biofilm-associated infections [73], [74], [75], [76], [77], [78], [79].

Epidemiology and clinical burden of biofilm-associated HAIs

Biofilm-associated infections account for up to 80% of all bacterial infections globally, with 60–70% of nosocomial infections directly linked to biofilm formation [80], [81], [82]. WHO and Centers for Disease Control and Prevention surveillance highlight increasing prevalence in ICU settings, where biofilm-forming pathogens like MRSA and *E. coli* exhibit MDR rates ranging from 17.9% to 100% [83]. The burden is amplified in patients with diabetes, chronic kidney disease, and multiple comorbidities, where biofilm-driven infections prolong ICU stay, escalate treatment costs, and increase mortality [2], [83].

Therapeutic roadblocks: why conventional therapy fails

Empirical monotherapy, a mainstay of early HAI management, fails against biofilm-embedded pathogens due to

Table 1: Diagnostic gaps and limitations of biofilm detection methods

Method	Diagnostic gaps	Limitations
Conventional culturing [66], [213], [214]	Often misses slow-growing/dormant biofilm cells; cannot detect fastidious or mixed-species communities	False negatives due to persisters; requires invasive sampling; heterogeneity of biofilm distribution
Molecular methods (PCR, WGS, metagenomics) [214], [215]	Cannot distinguish viable vs dead cells; may not differentiate planktonic vs biofilm phenotype	Contamination risk; lack of phenotype-specific markers; high cost and expertise required for WGS
Biomarker-based detection (host markers like α-defensin; bacterial Bap, EPS, cellulose, alginate) [66]	Many markers not biofilm-specific; variable expression across species/strains	Cross-reactivity with host factors; limited validation in clinical cohorts; lack of universal biofilm marker
Imaging (CLSM, FISH, SEM, TEM, MSI, Raman spectroscopy, NIR, µCT, PET) [66], [216], [217]	Invasive/destructive in many cases; often ex vivo	Low resolution for small biofilm aggregates (10–100 µm); need biofilm-specific contrast/labels; limited clinical applicability
Bio-impedance sensing (EIS, CMOS chips, smart catheters) [66]	Early-stage but promising; limited *in vivo* validation	Device integration challenges; requires miniaturization & standardization; signal interpretation still evolving
SAW [66], [216], [217], [218]	Still experimental; mostly tested on surfaces and fluids	Limited translation to *in vivo* implants; sensitivity depends on device-material interaction; no standardized clinical use
Other advanced biosensors (optical fibre, SPR, cantilevers) [66]	High sensitivity in lab; potential for multiplexing	Not yet validated for direct patient use; require stable surface coatings; complex fabrication; lack of regulatory approval

PCR: Polymerase chain reaction, WGS: Whole genome sequencing, CLSM: Confocal laser scanning microscopy, FISH: Fluorescence in situ hybridization, SEM: Scanning electron microscopy, TEM: Transmission electron microscopy, MSI: Mass spectrometry imaging, NIR: Near-infrared spectroscopy, µCT: Micro-computed tomography, PET: Positron Emission Tomography, EIS: Electrochemical impedance spectroscopy, SAW: Surface acoustic wave, SPR: Surface plasmon resonance

their fortified multicellular architecture [84]. Encased in a dense extracellular matrix, these bacteria exhibit up to 1,000-fold increased antibiotic tolerance via impaired drug penetration, metabolic dormancy, and phenotypic persistence [85]. Furthermore, biofilms act as genetic reservoirs for horizontal gene transfer, accelerating the spread of antimicrobial resistance across hospital ecosystems [86]. These adaptive mechanisms collectively render monotherapy ineffective – driving chronicity, relapse, and therapeutic failure, thus mandating a shift toward precision diagnostics and multi-targeted interventions [87].

Sub-inhibitory antibiotic exposure – a hidden catalyst of biofilm growth

Paradoxically, antibiotics may aggravate biofilm-associated infections [88]. Sub-inhibitory concentrations (sub-MICs), commonly encountered in poorly perfused tissues or between dosing intervals, not only facilitates biofilm maturation but can also modulate virulence, surface properties, and gene expression [89]. Notably, cell wall-active agents such as ampicillin and vancomycin induce envelope stress and enhance extracellular DNA (eDNA) release, reinforcing biofilm architecture [90]. Moreover, sub-MIC exposure can drive genetic adaptation through increased mutation, recombination, and horizontal gene transfer, thereby facilitating persistence and antimicrobial resistance [91].

Discussion

Anti-biofilm preventive strategies in clinical use

Antibacterial and antifouling coatings

Surface coatings incorporating antibiotics, antiseptics, hydrophilic polymers, silver nanoparticles, and antimicrobial peptides inhibit initial microbial adhesion and early biofilm formation [92]. Polihexanide covalently bound to a titanium alloy is bactericidal against both Gram-positive and Gram-negative bacteria. Modelling suggests that concentrations effective for antiseptic purposes are maintained near the surface for periods exceeding 6 months. Crucially, the polyhexanide film has no adverse effects on MG63 cells within a 48-hour cell culture. Across all measurement time points, viability and proliferation in the uncoated control group typically ranged from ≥90–95% with unchanged collagen synthesis. Within 15 minutes, the initial adhesion and spreading of osteoblasts on the test specimens were promoted [93], [94], [95], [96], [97]. Nanomaterials, with high surface-to-volume ratio and tunable physicochemical properties, disrupt biofilm structure, enhance antibiotic penetration, and reduce resistance development [98], [99]. These coatings act via release-based or contact-killing mechanisms while also preventing protein and cellular deposition critical for biofilm initiation [100], [101], [102]. Nanocoatings (e.g.,

nanosilver, titanium, copper, zinc oxide) show promise but face challenges in biocompatibility and long-term safety [103], [104]. Advances in lipid-coated nanoparticles, polymer-functionalized surfaces, and hydrogel or biodegradable alloy coatings have improved drug delivery and surface compatibility [38], [105], [106], [107]. Recent advances in cell membrane-coated nanocarriers illustrate the versatility of lipid nanotechnology in enhancing implant safety and performance [108]. Emerging systems including cell membrane-coated nanocarriers, enzyme-functionalized nanoparticles, and biofilm-responsive platforms, enable targeted EPS disruption and improved efficacy against polymicrobial biofilms, although approaches like nanoscale bacterial debridement remain experimental [109], [110], [111]. Emerging approaches like "nanoscale bacterial debridement", selectively detaching bacteria from biofilms, hold future potential but require further investigation [112].

Surface engineering to resist adhesion

Antiadhesive surfaces reduce bacterial-surface interactions, allowing microorganisms to be easily removed before biofilm maturation [113]. Techniques include anchoring polymer brushes to device surfaces using barnacle cement or polydopamine [114]. Such modifications on stainless steel have shown reduced protein adsorption and robust stability [115]. Tannic acid–based bifunctional coatings further enhance biofouling resistance [116].

Antimicrobial hydrogel and alloy coatings

Hydrogel coatings and antibacterial surface modifications have demonstrated efficacy in preventing orthopedic implant-associated infections [117], [118]. In parallel, biodegradable metal alloys with intrinsic antimicrobial properties are emerging as viable alternatives to conventional implants [119]. These innovations reduce microbial colonization and offer promise for clinical translation pending further validation.

Surface modification of biomaterials

Modifying implant surface properties (e.g., energy, roughness, hydrophilicity) without coatings has shown to reduce bacterial adhesion [120]. By altering surface characteristics, these strategies prevent bacterial settlement and biofilm initiation [121]. Techniques like pulsed laser evaporation help engineer inherently anti-adhesive surfaces.

Natural products in biofilm control: emerging strategies beyond antibiotics

Natural products inhibit biofilms by targeting adhesion, QS, and maturation [7]. Plant-derived compounds (flavonoids, terpenoids, and phenolics) disrupt biofilms by inhibiting surface attachment and QS without direct bactericidal action [122].

Phytochemicals like emodin, curcumin, and citrus flavonoids reduce virulence and biofilm integrity [123]. Natural agents like honey and cranberry proanthocyanidins further reduce mature biofilm biomass [124], [125]. Probiotic strains, especially *Lactobacillus* spp., further inhibit adhesion and destabilize biofilms via bacteriocins and biosurfactants [19].

Next-generation antimicrobials (NGAs) – disrupting medical device biofilms

NGAs combat medical device biofilms through multi-targeted mechanisms that overcome AMR [126]. Enzymatic agents such as DNase I and proteases (e.g., Proteinase K, dispersin B) degrade EPS components, promoting biofilm dispersal and enhancing antibiotic penetration [127], [128], [129], [130]. Anti-adhesion strategies (pilicides, glycomimetic) prevent initial bacterial attachment while while QS inhibitors disrupt biofilm maturation. Additionally, cyclic di-GMP modulators and nitric oxide donors induce biofilm dispersal via intracellular signaling pathways [126], [131], [132], [133].

Quorum sensing inhibition and biofilm disruption

Targeting QS pathways with small molecules, enzymes (e.g., DNase I), or natural extracts (e.g., rosmarinic acid, ginger) can prevent biofilm formation [134], [135]. These methods interfere with bacterial communication and matrix integrity, thereby impairing biofilm stability and maturation [121]. NSAIDs (e.g., meloxicam, aspirin) and antibiotics (e.g., azithromycin, ciprofloxacin) exhibit QS inhibitors activity [136]. Synergistic combinations, such as resveratrol with aminoglycosides, enhance biofilm disruption and prevent QS inhibitors resistance [137]. Adjuncts like D-amino acids and proteases enhance antibiotic efficacy against resistant biofilms [138], [139].

Plant-based strategies for control of biofilm associated infections

Phytochemicals (phenolics, flavonoids, terpenoids, alkaloids, essential oils) act via QS inhibition, membrane disruption, EPS degradation, and nutrient sequestration. They are eco-friendly, often synergistic, and less prone to resistance development [140].
Examples include *Hypericum lydium* extracts (anti-MRSA activity), *Cochlospermum regium* (phenol-rich) against MRSA biofilms, *Persea americana* seed extracts (enhanced wound healing and antibiofilm effect), isoflavonoid- and xanthone-rich *Iris pseudacorus, Curcuma aromatica* flavonoids/alkaloids for Gram-positive biofilms and *Frangula angus* and *Hymenocallis littoralis* with broad-spectrum antibiofilm activity.

Nanotechnology-based strategies for control of biofilm associated infections

Nanoparticles (NPs) offer high surface reactivity, biofilm penetration, and multi-mechanistic antimicrobial action (membrane disruption, ROS generation, DNA damage) [140]. Examples are:

- Silver NPs (AgNPs) are effective suture/device coatings with broad-spectrum action; TiO_2–Ag hybrids zwitterionic AgNP dressings works by ROS generation (TiO_2) with AgNP bactericidal action [140].
- Composite and functionalized NPs such as amphora-shaped porous Ti implants deters bacterial attachment, CaP–Ag coatings prevent adhesion on bone implants and release bactericidal Ag^+ for orthopedic devices [140].
- Other nanomaterials are polymeric NPs, dendrimers, and liposomes for targeted delivery and improved biofilm penetration [140]

However, translation into clinical practice remains limited due to concerns regarding long-term toxicity, scalability, regulatory approval, and cost-effectiveness, particularly in low-resource healthcare settings [141].

Established and clinically applicable therapeutic anti-biofilm approaches

Physical chemical and biological approaches

Physical methods such as ionizing/UV radiation and ultrasonic cavitation disrupt biofilms through mechanical and oxidative stress [142]. Cold atmospheric plasma enhances eradication through reactive oxygen species (ROS)/reactive nitrogen species, mediated damage, with synergistic effects when combined with ultrasound [143]. Microneedles further improve antimicrobial penetration by breaching the EPS matrix [144]. In contrast, chemical agents are limited by toxicity, instability, and resistance, whereas biological strategies, such as enzymes, and bacteriophages, offer greater specificity and safety [145].

Bacteriocins

These are ribosomally synthesized antimicrobial peptides (AMPs), have emerged as promising antibiofilm agents with multifaceted mechanisms [146], [147]. They inhibit bacterial adhesion and biofilm formation, reduce EPS production, and disrupt mature biofilms [146], [148]. Studies demonstrate significant reductions in biofilm biomass and matrix integrity across key pathogens, including *S. aureus, P. aeruginosa*, and *E. faecalis* [149]. Additionally, bacteriocins can eradicate biofilm-associated cells, highlighting their potential in managing biofilm-mediated healthcare-associated infections [150].

Conventional management of biofilm-associated infections

Biofilm-associated infections require combined surgical and antimicrobial management. Superficial infections may respond to drainage, whereas deep or device-related infections often require debridement, prosthesis removal, and targeted therapy [151]. Negative pressure wound therapy augments debridement by promoting granulation and reducing bioburden [152]. Antibiotic-loaded spacers (e.g., vancomycin or gentamicin) with rifampin-fluoroquinolone regimens improve eradication of staphylococcal biofilms, whereas resistant infections may require agents such as linezolid, daptomycin, tigecycline, or carbapenems [140]. Polymicrobial biofilms further complicate treatment by accelerating tissue damage and reducing antibiotic efficacy [153].

Antimicrobial peptides (AMPs)

AMPs disrupt bacterial membranes, suppress biofilm-related gene expression, and modulate QS, offering potent activity against both planktonic and biofilm-embedded pathogens [154]. Peptides like LL-37 and lactoferrin derivatives exhibit broad-spectrum antibiofilm effects, particularly in respiratory infections, and synergize with antibiotics to enhance efficacy [155], [156]. Lactoferrin, an innate immune component, prevents *P. aeruginosa* biofilm formation by stimulating bacterial twitching motility [10]. Metal-binding AMPs, such as Gaduscidin-1, are effective in hostile microenvironments, including those seen in *P. aeruginosa* biofilms [157], [158].

Established biological therapies

Bacteriophage therapy

Bacteriophages disrupt biofilms through enzymatic degradation (e.g., haemolysinase, depolymerases) and lytic replication, effectively targeting both antibiotic-sensitive and resistant bacteria [10], [159], [160]. Specific phages (e.g., Φ15, Φ29, PD1, PE2, T4) have demonstrated efficacy against Pseudomonas, Staphylococcus, Salmonella, and Klebsiella biofilms [161], [162]. Phage-antibiotic combinations (e.g., T4 with tobramycin, or phage with amoxicillin) enhance biofilm eradication and limit resistance emergence [163]. Phage cocktails further expand host range and reduce resistance development [164].

Enzyme-based disruption of bacterial biofilms

Enzymes (oxidases, proteases, and polysaccharide-degrading hydrolases) disrupt biofilms by degrading EPS, interfering with QS, and inhibiting maturation [164], [165]. Their high specificity makes them ideal biological antibiofilm agents. However, their stability is limited; immobilization strategies, such as crosslinked enzyme aggregates (CLEA), enhance stability and reusability [166]. Magnetic CLEA formulation (m-combi-CLEA) have shown >75% in-

hibition of *E. coli and S. aureus* biofilms, highlighting their therapeutic potential [167].

Vaccines

Vaccines incorporating biofilm-derived antigens represent a promising strategy to enhance protection against persistent infections [168]. In *Bordetella pertussis*, biofilm-derived outer membrane vesicles induce stronger immunogenicity and protection than planktonic counterparts, including against pertactin-deficient strains, and generate durable mucosal $CD4^+$ memory responses [169], [170]. While still under investigation, biofilm-based vaccines offer a compelling direction for future anti-biofilm immunotherapies [14].

Monoclonal antibodies as therapeutics for fungal biofilm infections

Monoclonal antibodies (MAb) targeting biofilm-specific antigens enable radioimmunotherapy through MAb-guided alpha radiation, allowing in situ treatment when device removal is contraindicated [171]. Prophylactic MAb administration prevents Cryptococcal biofilm establishment post-implantation [172]. Chitosan device coatings provide biocompatible protection by disrupting microbial membrane integrity and preventing surface colonization [173]. *In vivo* studies demonstrate efficacy against *Candida* biofilms on CVCs without host cell toxicity [174]. Optimized antifungal approaches – targeting early biofilm stages, novel formulations (amphotericin B lipid complex), and combination therapies – significantly enhance treatment efficacy compared to conventional methods [175].

Emerging and advanced therapeutic strategies

The growing threat of antimicrobial resistance underscores the need for novel approaches.

Energy-based methods

Electric fields (e.g., DC, AC, pulsed electric fields) induce membrane disruption and enhance antibiotic uptake through a "bioelectric effect" [176], [177]. These approaches help destabilize the biofilm matrix and enhance drug penetration into biofilm-embedded bacteria [121]. In vitro and animal studies show synergy with antibiotics, reducing biofilm burden, but further clinical validation is needed [178].

Low-frequency ultrasound enhances antibiotic penetration and disrupts EPS matrices. Devices delivering surface acoustic waves weakens biofilm cohesion and promotes antimicrobial access and have demonstrated >85% reduction in common pathogens' biofilms when combined with antimicrobials [179]. Although ultrasound enhances drug penetration, its clinical applicability is limited by optimization challenges, device standardization, and gaps in clinical translation [180].

Antimicrobial photodynamic therapy (aPDT) leverages light-activated photosensitizers to generate ROS and directly damages biofilm components and microbial cells, leading to effective biofilm disruption [121]. Laser and LED-based systems (405–940 nm) have successfully eradicated biofilms on various medical substrates. Clinical use is constrained by limited light penetration, challenges in effective photosensitizer delivery, and difficulty in treating deep-seated infections, with further safety data required for clinical translation [181], [182], [183].

Molecular and genetic tools

CRISPR/Cas systems enable precise disruption of biofilm formation by targeting key regulatory genes, such as *icaA* in *S. aureus*, *lasR/rhlR* in *P. aeruginosa*, and *pelA* in *P. aeruginosa* and *E. coli*, thereby reducing biofilm biomass and adhesion [184], [185], [186], [187]. Additionally, CRISPR-associated nucleases can selectively degrade AMR determinants, re-sensitizing biofilm-embedded pathogens to conventional antibiotics [188]. To overcome delivery barriers, engineered bacteriophages carrying CRISPR constructs have shown efficacy against *K. pneumoniae* biofilms, while nanocarrier and liposomal systems further enhance penetration and therapeutic efficiency within the biofilm matrix [189]. Clinical translation is hindered by delivery barriers, off-target effects, biosafety concerns, and complex regulatory approval pathways [190].

Aptamers, synthetic single-stranded oligonucleotides or peptides, exhibit high-affinity, target-specific binding via defined 3D structures [191]. They disrupt biofilms by depolarizing bacterial membranes and enhancing antibiotic delivery. Aptamer-functionalized nanomaterials, such as aptamer-graphene oxide complexes, have shown >90% inhibition of *Salmonella typhimurium* biofilms [192]. Their therapeutic application is limited by rapid degradation *in vivo*, poor stability, and challenges in targeted delivery and large-scale production [193].

Peptide nucleic acids (PNAs) bind bacterial DNA with high specificity and affinity, showing promise against MDR pathogens and biofilms [194]. Although limited by poor penetration, delivery strategies such as conjugation with cell-penetrating peptides enhance efficacy. PNAs targeting *ftsZ, efaA*, or *acpP* genes inhibit bacterial division and biofilm formation in *Escherichia coli, Enterococcus* spp., and *Hemophilus influenzae* [195], [196], [197]. Synergistic combinations with antibiotics (e.g., polymyxin B) potentiate anti-biofilm effects, making PNAs a compelling NGA strategy [198]. PNAs face significant barriers including poor cellular uptake, delivery challenges, and potential toxicity at higher concentrations [199].

Real-time biofilm detection via biosensors (e.g., impedance-based systems, Raman spectroscopy, qPCR) allows for early identification enabling timely therapeutic intervention before biofilm maturation, improving treatment outcomes [121], [200], [201]. Techniques like SERS and interdigitated microelectrodes offer high sensitivity for detecting pathogens on medical surfaces [121]. Despite

Table 2: Biofilm-eradicating agents with their action

Category	Representative agents / examples	Action on biofilms	Experimental studies
Polymyxins/ conventional antibiotics	Colistin (polymyxin E); combinations with rifampicin, doripenem, tigecycline, etc.	Disrupts Gram-negative outer membrane, can kill metabolically inactive cells in inner biofilm layers; often used in high dose or combination to overcome tolerance.	Clinical/use & experimental studies on colistin vs biofilms [219], [220], [221]
MITO	Daptomycin ± rifampicin (for MRSA/device infections)	Membrane depolarization and bactericidal activity against adherent cells; rifampicin synergy improves activity against biofilm-embedded staphylococci.	*In vitro* and implant-associated infection studies with daptomycin ± rifampin [222], [223].
Quorum-sensing inhibitors/ macrolides	Azithromycin; synthetic furanones and other QS antagonists	Interfere with QS→ reduce virulence factor production, inhibit biofilm formation and maintenance, sensitize bacteria to host/antibiotics.	Azithromycin as a QS inhibitor (P. aeruginosa) and QS inhibitor [224], [225].
Matrix-degrading enzymes	DNase I (targets eDNA); Dispersin B (glycoside hydrolase)	Degrade eDNA or polysaccharide matrix → detach biofilm, increase antibiotic penetration and immune access.	Experimental studies of DNase I & Dispersin B [226].
Antimicrobial peptides	LL-37, pexiganan, engineered antibiofilm peptides (DJK-5, etc.)	Disrupt membranes, modulate QS and attachment, prevent/or disperse biofilm at sub-MIC concentrations; often synergize with antibiotics.	LL-37 antibiofilm work and AMP studies [227], [228].
Metal / inorganic nanoparticles	AgNPs, chitosan NPs, others	Physical disruption, generation of ROS, release of metal ions that damage cells and EPS; can penetrate/disrupt matrix.	AgNPs as antibiofilm agents and safety/efficacy discussions [225], [229].
Natural products / phytochemicals	Flavonoids (quercetin, baicalein), terpenoids, phenolics	Inhibit adhesion, interfere with EPS synthesis and QS, reduce biofilm biomass; many studied *in vitro* and some *in vivo* models.	Experimental work on flavonoids' antibiofilm effects [230], [231].
Physical / device-directed methods	Ultrasound (sonication), aPDT	Mechanical disruption (ultrasound) or photosensitizer + light → ROS generation and local killing/disruption of biofilm.	aPDT and ultrasound applications for localized infections [232], [233].
Anti-adhesive / antimicrobial coatings	Silver-alloy / silver-coated catheters, hydrophilic / antibiotic-impregnated polymers	Prevent initial adhesion and early biofilm establishment; some reduce DAI rates in trials, results variable.	Clinical trials on silver-coated catheters and coatings [234], [235].
Chelators and matrix destabilizers	EDTA, citrate (used topically or in lock solutions)	Chelate divalent cations stabilizing EPS → destabilize matrix and enhance antibiotic penetration or mechanical removal.	Studies showing EDTA enhances antibiotic activity and biofilm disruption [236].

high sensitivity, widespread clinical use is limited by high cost, need for specialized infrastructure, and challenges in real-time clinical integration [180].

Targeted molecular anti-biofilm strategies

Catabolite control protein A (CcpA) is a key regulator of biofilm formation in *S. aureus*, promoting adhesin and eDNA production (cidA/icaA) while repressing the sak gene, which encodes staphylokinase and enhancing virulence via a-hemolysin [202]. Inhibiting CcpA–DNA binding reduces toxin expression against *S. aureus* biofilm-related infections, highlighting the CcpA–Sak axis as a promising low-toxicity therapeutic potential [203], [204]. Functional amyloids play a central role in biofilm development across several bacterial species [10]. Small molecules such as FN075 and BibC6 in *E. coli*, and AA-861 or parthenolide in *Bacillus subtilis*, inhibit amyloid fiber formation or disrupt established biofilms, thereby reducing virulence [205], [206]. These findings highlight that targeting amyloid assembly can effectively weaken biofilm structure and persistence. Table 2 summarizes all biofilm-eradicating agents and their action.

Clinical translation and real-world challenges

Although all these strategies demonstrate significant antibiofilm activity in vitro and in preclinical models, robust clinical evidence supporting their routine use remains limited, highlighting a critical gap between experimental innovation and bedside application [207], [208].

Most approaches, including NPs, AMP, QS inhibitors, and enzymatic therapies, are supported primarily by in vitro or animal model data, with a paucity of large-scale randomized clinical trials [209], [210]. Current management of biofilm-associated HAIs continues to rely heavily on device removal, prolonged antimicrobial therapy, and infection control measures [51].

Cost, scalability, toxicity, and regulatory barriers further hinder clinical adoption, particularly in low- and middle-income countries where the burden of HAIs is highest [141], [211]. Even promising interventions such as antimicrobial coatings and lock therapies have demonstrated variable efficacy in clinical settings. Bridging this gap requires well-designed clinical trials, standardized evaluation models, and integration of biofilm-specific strategies into existing infection control frameworks [212].

Conclusion

Biofilms are a critical yet often underrecognized driver of HAIs, contributing to persistence, recurrence, and antimicrobial resistance. Through coordinated processes such as adhesion, extracellular matrix formation, and quorum sensing, biofilm-embedded pathogens evade host defenses and exhibit marked tolerance to conventional antimicrobial therapies. As a result, standard treatment strategies designed for planktonic organisms are frequently ineffective, particularly in device-associated infections such as CLABSI, CAUTI, VAP, and SSI.

The intrinsic tolerance conferred by the biofilm matrix, metabolic heterogeneity, and persister cell populations necessitates a paradigm shift toward biofilm-targeted prevention, early detection, and multi-modal treatment approaches. Preventive strategies particularly surface engineering, antimicrobial coatings, and quorum sensing inhibition, offer the most effective opportunity to reduce biofilm establishment, especially in device-associated settings.

Therapeutically, while established approaches such as surgical debridement, device removal, and combination antimicrobial therapy remain the clinical cornerstone, a broad spectrum of emerging interventions, including nanotechnology-based systems, antimicrobial peptides, bacteriophages, enzyme-based matrix disruption, and molecular tools such as CRISPR – demonstrate promising anti-biofilm activity. However, a critical translational gap persists, as most of these strategies remain confined to in vitro and preclinical models, with limited validation in large-scale clinical trials.

Importantly, this review underscores the need for integration of biofilm-specific diagnostics, preventive strategies, and targeted therapeutics into existing infection control frameworks. Advancing the field will require standardized models for biofilm evaluation, robust clinical studies, and interdisciplinary collaboration bridging microbiology, material science, and clinical medicine.

Addressing biofilm-associated HAIs demands a shift from reactive treatment to proactive prevention and precision-targeted therapy. Bridging the gap between mechanistic understanding and clinical application is essential to reduce the burden of HAIs, combat AMR, and improve patient outcomes in modern healthcare systems.

Notes

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References

1. Lowe H, Woodd S, Lange IL, Janjanin S, Barnet J, Graham W. Challenges and opportunities for infection prevention and control in hospitals in conflict-affected settings: a qualitative study. Confl Health. 2021 Dec 20;15(1):94. DOI:10.1186/s13031-021-00428-8
2. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. Microorganisms. 2023 Jun 19;11(6):1614. DOI: 10.3390/microorganisms11061614
3. Tran PL, Lowry N, Campbell T, Reid TW, Webster DR, Tobin E, Aslani A, Mosley T, Dertien J, Colmer-Hamood JA, Hamood AN. An organoselenium compound inhibits Staphylococcus aureus biofilms on hemodialysis catheters in vivo. Antimicrob Agents Chemother. 2012 Feb;56(2):972-8. DOI: 10.1128/AAC.05680-11
4. Donlan RM. Biofilms and device-associated infections. Emerg Infect Dis. 2001 Mar-Apr;7(2):277-81. DOI: 10.3201/eid0702.010226
5. Anand G, Lahariya R, Priyadarshi K, Sarfraz A. Emerging threat of WHO priority pathogens in ICU-associated CLABSI and CAUTI: an integrated analysis of resistance patterns, epidemiological trends, and stewardship strategies. Acta Clin Belg. 2025 Oct;80(5):135-45. DOI: 10.1080/17843286.2025.2546420

6. Pan Y, Song S, Tang X, Ai Q, Zhu D, Liu Z, Yu J. Streptococcus sp. in neonatal endotracheal tube biofilms is associated with ventilator-associated pneumonia and enhanced biofilm formation of Pseudomonas aeruginosa PA01. Sci Rep. 2017 Jun 13;7(1):3423. DOI: 10.1038/s41598-017-03656-2

7. Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol. 2010 Aug 1;59(3):227-38. DOI: 10.1111/j.1574-695X.2010.00665.x

8. Highmore CJ, Melaugh G, Morris RJ, Parker J, Direito SOL, Romero M, Soukarieh F, Robertson SN, Bamford NC. Translational challenges and opportunities in biofilm science: a BRIEF for the future. NPJ Biofilms Microbiomes. 2022 Aug 29;8(1):68. DOI: 10.1038/s41522-022-00327-7

9. Lahariya R, Anand G. Early detection of central line-associated bloodstream infection in intensive care unit patients using the systemic inflammatory response index (SIRI). GMS Hyg Infect Control. 2025;20:Doc77. DOI: 10.3205/dgkh000606

10. Wu H, Moser C, Wang HZ, Høiby N, Song ZJ. Strategies for combating bacterial biofilm infections. Int J Oral Sci. 2015 Mar 23;7(1):1-7. DOI: 10.1038/ijos.2014.65

11. Paredes J, Alonso-Arce M, Schmidt C, Valderas D, Sedano B, Legarda J, Arizti F, Gómez E, Aguinaga A, Del Pozo JL, Arana S. Smart central venous port for early detection of bacterial biofilm related infections. Biomed Microdevices. 2014 Jun;16(3):365-74. DOI: 10.1007/s10544-014-9839-3

12. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019 May 16;8:76. DOI: 10.1186/s13756-019-0533-3

13. Inoue H. Strategic approach for combating antimicrobial resistance (AMR). Glob Health Med. 2019 Dec 31;1(2):61-4. DOI: 10.35772/ghm.2019.01026

14. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. 2023 Apr 6;13:1137947. DOI: 10.3389/fcimb.2023.1137947

15. Zafer MM, Mohamed GA, Ibrahim SRM, Ghosh S, Bornman C, Elfaky MA. Biofilm-mediated infections by multidrug-resistant microbes: a comprehensive exploration and forward perspectives. Arch Microbiol. 2024 Feb 14;206(3):101. DOI: 10.1007/s00203-023-03826-z

16. Anand G, Lahariya R, Sarfraz A, Thakuria B, Kokkayil P, Pati BK. Antimicrobial resistance profiles and mortality rates in intensive care unit patients having central line associated blood stream infection: a temporal analysis. GMS Hyg Infect Control. 2026 Mar 2;21:Doc24. DOI: 10.3205/dgkh000633

17. Hall-Stoodley L, Costerton J, Stoodley P. Bacterial biofilms: from the Natural environment to infectious diseases. Nat Rev Microbiol. 2004 Feb;2(2): 95-108. DOI: 10.1038/nrmicro821

18. Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev. 2017 May 1;41(3):276-301. DOI: 10.1093/femsre/fux010

19. Oluwole OM. Biofilm: formation and natural products' approach to control - a review. Afr J Infect Dis. 2022 Aug 17;16(2 Suppl):59-71. DOI: 10.21010/Ajid.v16i2S.7

20. Zhang M, Han W, Gu J, Qiu C, Jiang Q, Dong J, Lei L, Li F. Recent advances on the regulation of bacterial biofilm formation by herbal medicines. Front Microbiol. 2022 Nov 8;13:1039297. DOI: 10.3389/fmicb.2022.1039297

21. Cui S, Kim E. Quorum sensing and antibiotic resistance in polymicrobial infections. Commun Integr Biol. 2024 Oct17;17(1):2415598. DOI: 10.1080/19420889.2024.2415598

22. Verderosa AD, Totsika M, Fairfull-Smith KE. Bacterial biofilm eradication agents: A current review. Front Chem. 2019 Nov 28;7:824. DOI: 10.3389/fchem.2019.00824

23. Powell LC, Abdulkarim M, Stokniene J, Yang QE, Walsh TR, Hill KE, Gumbleton M, Thomas DW. Quantifying the effects of antibiotic treatment on the extracellular polymer network of antimicrobial resistant and sensitive biofilms using multiple particle tracking. Npj Biofilms Microbiomes. 2021 Feb 5;7(1):13. DOI: 10.1038/s41522-020-00172-6

24. Bordi C, de Bentzmann S. Hacking into bacterial biofilms: a new therapeutic challenge. Ann Intensive Care. 2011 Jun 13;1(1):19. DOI: 10.1186/2110-5820-1-19

25. Costa-Orlandi CB, Sardi JCO, Pitangui NS, de Oliveira HC, Scorzoni L, Galeane MC, Medina-Alarcón KP, Melo WCMA, Marcelino MY, Braz JD, Fusco-Almeida AM, Mendes-Giannini MJS. Fungal Biofilms and Polymicrobial Diseases. J Fungi (Basel). 2017 May 10;3(2):22. DOI: 10.3390/jof3020022

26. Martinez LR, Fries BC. Fungal biofilms: Relevance in the setting of human disease. Curr Fungal Infect Rep. 2010 Dec 1;4(4):266-75. DOI: 10.1007/s12281-010-0035-5

27. Prinzi A, Rohde R. The role of bacterial biofilms in antimicrobial resistance. ASM.org. 2023. Available from: https://asm.org/Articles/2023/March/The-Role-of-Bacterial-Biofilms-in-Antimicrobial-Re

28. Rottier W, Seidelman J, Wouthuyzen-Bakker M. Antimicrobial treatment of patients with a periprosthetic joint infection: basic principles. Arthroplasty. 2023 Mar 2;5(1):10. DOI: 10.1186/s42836-023-00169-4

29. Werneburg GT, Hettel D, Goldman HB, Vasavada SP, Miller AW. Indwelling urological device biofilm composition and characteristics in the presence and absence of infection. Urology. 2025 Feb;196:82-9. DOI: 10.1016/j.urology.2024.10.021

30. Bouhrour N, Nibbering PH, Bendali F. Medical device-associated biofilm infections and multidrug-resistant pathogens. Pathogens. 2024 May 8;13(5):393. DOI: 10.3390/pathogens13050393

31. Singhai M, Malik A, Shahid M, Malik MA, Goyal R. A study on device-related infections with special reference to biofilm production and antibiotic resistance. J Glob Infect Dis. 2012 Oct;4(4):193-8. DOI: 10.4103/0974-777X.103896

32. Harriott MM, Noverr MC. Candida albicans and Staphylococcus aureus form polymicrobial biofilms: effects on antimicrobial resistance. Antimicrob Agents Chemother. 2009 Sep;53(9):3914-22. DOI: 10.1128/AAC.00657-09

33. Tabassum N, Jeong GJ, Jo DM, Khan F, Kim YM. Treatment of Staphylococcus aureus and Candida albicans polymicrobial biofilms by phloroglucinol-gold nanoparticles. Microb Pathog. 2023 Dec;185:106416. DOI: 10.1016/j.micpath.2023.106416

34. Giordano V, Giannoudis PV. Biofilm Formation, antibiotic resistance, and infection (BARI): The triangle of death. J Clin Med. 2024 Jan;13(19):5779. DOI: 10.3390/jcm13195779

35. Li P, Yin R, Cheng J, Lin J. Bacterial biofilm formation on biomaterials and approaches to its treatment and prevention. Int J Mol Sci. 2023 Jan;24(14):11680. DOI: 10.3390/ijms241411680

36. Weber DJ, Rutala WA, Anderson DJ, Sickbert-Bennett EE. Biofilms on medical instruments and surfaces: Do they interfere with instrument reprocessing and surface disinfection. Am J Infect Control. 2023 Nov;51(11S):A114-9. DOI: 10.1016/j.ajic.2023.04.158

37. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. 2023 Apr 6;13:1137947. DOI: 10.3389/fcimb.2023.1137947

38. Mishra A, Aggarwal A, Khan F. Medical Device-associated infections caused by biofilm-forming microbial pathogens and controlling strategies. Antibiotics. 2024 Jul 4;13(7):623. DOI: 10.3390/antibiotics13070623

39. Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018 Dec 28;4(12):e01067. DOI: 10.1016/j.heliyon.2018.e01067

40. Zou J, Peng B, Qu J, Zheng J. Are bacterial persisters dormant cells only? Front Microbiol. 2022 Feb 2;12:708580. DOI: 10.3389/fmicb.2021.708580

41. Damyanova T, Paunova-Krasteva T. What we still don't know about biofilms—current overview and key research information. Microbiol Res. 2025 Feb;16(2):46. DOI: 10.3390/microbiolres16020046

42. Assefa M, Amare A. Biofilm-Associated multi-drug resistance in hospital-acquired infections: A review. Infect Drug Resist. 2022 Jan 1;15:5061-8. DOI: 10.2147/IDR.S379502

43. Anand G, Sarfraz A, Thakuria B, Kokkayil P, Pati B. Incidence, microbial profile and antimicrobial resistance trends of CLABSI in adult ICUs: a longitudinal prospective study. Iran J Microbiol. 2026;18(1):41-7. DOI: 10.18502/ijm.v18i1.20905

44. Fang L, Qiao Y, Li X, Wang C, Li C, Luan T, Wang W. A new dynamic in vitro model for evaluating antimicrobial activity against bacterial biofilms on central venous catheters. Microbiol Spectr. 2024;12(9):e00237-24. DOI: 10.1128/spectrum.00237-24

45. Ali A, Zahra A, Kamthan M, Husain FM, Albalawi T, Zubair M, Alatawy R, Abid M, Noorani MS. Microbial Biofilms: Applications, Clinical Consequences, and Alternative Therapies. Microorganisms. 2023 Jul 29;11(8):1934. DOI: 10.3390/microorganisms11081934

46. Craven DE, Steger KA. Epidemiology of nosocomial pneumonia: New perspectives on an old disease. CHEST. 1995 Aug 1;108(2):1S-16S. DOI: 10.1378/chest.108.2

47. Rello J, Soñora R, Jubert P, Artigas A, Rué M, Vallés J. Pneumonia in intubated patients: role of respiratory airway care. Am J Respir Crit Care Med. 1996 Jul;154(1):111-5. DOI: 10.1164/ajrccm.154.1.8680665

48. Taner F, Baddal B, Theodoridis L, Petrovski S. Biofilm production in intensive care units: Challenges and implications. Pathogens. 2024 Nov;13(11):954. DOI: 10.3390/pathogens13110954

49. Gil-Perotin S, Ramirez P, Marti V, Sahuquillo JM, Gonzalez E, Calleja I, Menendez R, Bonastre J. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. Crit Care. 2012;16(3):R93. DOI: 10.1186/cc11357

50. Codru IR, Vintilă BI, Sava M, Bereanu AS, Neamtu SI, Bădilă RM, Bîrlutiu V. Optimizing diagnosis and management of ventilator-associated pneumonia: A Systematic evaluation of biofilm detection methods and bacterial colonization on endotracheal tubes. Microorganisms. 2024 Sept 28;12(10):1966. DOI: 10.3390/microorganisms12101966

51. Adair CG, Gorman SP, Feron BM, Byers LM, Jones DS, Goldsmith CE, Moore JE, Kerr JR, Curran MD, Hogg G, Webb CH, McCarthy GJ, Milligan KR. Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intensive Care Med. 1999 Oct;25(10):1072-6. DOI: 10.1007/s001340051014

52. Agarwal J, Radera S. Biofilm-Mediated Urinary Tract Infections. In: Kumar S, Chandra N, Singh L, Hashmi MZ, Varma A, editors. Biofilms in Human Diseases: Treatment and Control. Cham: Springer Int Publ;2019 [cited 2025 Jun 8]. p. 177-213.DOI:10.1007/978-3-030-30757-8_13

53. Mirzaei R, Mohammadzadeh R, Alikhani MY, Shokri Moghadam M, Karampoor S, Kazemi S, Barfipoursalar A, Yousefimashouf R. The biofilm-associated bacterial infections unrelated to indwelling devices. IUBMB Life. 2020 Jul;72(7):1271-85. DOI: 10.1002/iub.2266

54. Caldara M, Belgiovine C, Secchi E, Rusconi R. Environmental, microbiological, and immunological features of bacterial biofilms associated with implanted medical devices. Clin Microbiol Rev. 35(2):e00221-20. DOI: 10.1128/cmr.00221-20

55. Tsikopoulos K, Meroni G. Periprosthetic Joint infection diagnosis: A narrative review. Antibiotics. 2023 Oct;12(10):1485. DOI: 10.3390/antibiotics12101485

56. Almasri D, Dahman Y. Prosthetic joint infections: Biofilm formation, management, and the potential of mesoporous bioactive glass as a new treatment option. Pharmaceutics. 2023 May;15(5):1401. DOI: 10.3390/pharmaceutics15051401

57. Wagner C, Hänsch GM. Pathophysiologie der implantatassoziierten Infektion : Vom Biofilm zur Osteolyse und septischen Lockerung [Pathophysiology of implant-associated infections: From biofilm to osteolysis and septic loosening]. Orthopade. 2015 Dec;44(12):967-73. DOI: 10.1007/s00132-015-3183-z.

58. James GA, Boegli L, Hancock J, Bowersock L, Parker A, Kinney BM. Bacterial adhesion and biofilm formation on textured breast implant shell materials. Aesthetic Plast Surg. 2019 Apr;43(2):490-7. DOI: 10.1007/s00266-018-1234-7

59. Asokan S, Pandey RK, Jalil MA, Alhussen SKA, Yousif SIA, Abbas RK, Vijayan S, Rajeswary D, Jacob T, Atiyah MM. Biofilm associated infections on medical devices: Pathogenesis, diagnostic challenges, and control strategies. Microbe. 2026 Jun 1;11:100712. DOI: 10.1016/j.microb.2026.100712

60. Esfahanizadeh N, Mirmalek SP, Bahador A, Daneshparvar H, Akhoundi N, Pourhajibagher M. Formation of biofilm on various implant abutment materials. Gen Dent. 2018 Sep-Oct;66(5):39-44.

61. Silva NBSb, Marques LA, Röder DDB. Diagnosis of biofilm infections: current methods used, challenges and perspectives for the future. J Appl Microbiol. 2021 Nov;131(5):2148-60. DOI: 10.1111/jam.15049

62. Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002 Apr;15(2):167-93. DOI: 10.1128/CMR.15.2.167-193.2002

63. Vertes A, Hitchins V, Phillips KS. Analytical challenges of microbial biofilms on medical devices. Anal Chem. 2012 May 1;84(9):3858-66. DOI: 10.1021/ac2029997

64. Donlan RM, Piede JA, Heyes CD, Sanii L, Murga R, Edmonds P, El-Sayed I, El-Sayed MA. Model system for growing and quantifying streptococcus pneumoniae biofilms in situ and in real time. Appl Environ Microbiol. 2004 Aug;70(8):4980-8. DOI: 10.1128/AEM.70.8.4980-4988.2004

65. Lahariya R, Anand G, Sarfraz A, Tiewsoh JBA, Kumar A. CLABpredICU—AI-driven risk prediction for CLABSI in intensive care units based on clinical and biochemical parameters. Am J Infect Control. 2025 Aug;53(8):875-80. DOI: 10.1016/j.ajic.2025.05.016

66. Xu Y, Dhaouadi Y, Stoodley P, Ren D. Sensing the unreachable: challenges and opportunities in biofilm detection. Curr Opin Biotechnol. 2020 Aug 1;64:79-84. DOI: 10.1016/j.copbio.2019.10.009

67. Dimauro G, Deperte F, Maglietta R, Bove M, La Gioia F, Renò V, Simone L, Gelardi M. A novel approach for biofilm detection based on a convolutional neural network. Electronics. 2020 Jun;9(6):6. DOI: 10.3390/electronics9060881

68. Wasilewski T, Kamysz W, Gębicki J. AI-Assisted detection of biomarkers by sensors and biosensors for early diagnosis and monitoring. Biosensors. 2024 Jul 22;14(7):356. DOI: 10.3390/bios14070356

69. Habib MB, Batool G, Shah NA, Muhammad T, Akbar NS, Shahid A. Biofilm-mediated infections; novel therapeutic approaches and harnessing artificial intelligence for early detection and treatment of biofilm-associated infections. Microb Pathog. 2025 Jun 1;203:107497. DOI: 10.1016/j.micpath.2025.107497

70. Alsulimani A, Akhter N, Jameela F, Ashgar RI, Jawed A, Hassani MA, Dar SA. The Impact of Artificial Intelligence on Microbial Diagnosis. Microorganisms. 2024 May 23;12(6):1051. DOI: 10.3390/microorganisms12061051

71. Abeyrathna D, Ashaduzzaman M, Malshe M, Kalimuthu J, Gadhamshetty V, Chundi P, Subramaniam M. An AI-based approach for detecting cells and microbial byproducts in low volume scanning electron microscope images of biofilms. Front Microbiol. 2022 Dec 1;13:996400. DOI: 10.3389/fmicb.2022.996400

72. Anand G, Lahariya R, Sarfraz A, Tiewsoh JBA. Early screening of central line-associated bloodstream infections: A novel comparative analysis of AISI, SII, and SIRI as predictive biomarkers. Am J Infect Control. 2025 Dec;53(12):1320-4. DOI: 10.1016/j.ajic.2025.08.020

73. Hannig C, Follo M, Hellwig E, Al-Ahmad A. Visualization of adherent micro-organisms using different techniques. J Med Microbiol. 2010;59(1):1-7. DOI: 10.1099/jmm.0.015420-0

74. Priester JH, Horst AM, Van De Werfhorst LC, Saleta JL, Mertes LAK, Holden PA. Enhanced visualization of microbial biofilms by staining and environmental scanning electron microscopy. J Microbiol Methods. 2007 Mar 1;68(3):577-87. DOI: 10.1016/j.mimet.2006.10.018

75. Mountcastle SE, Vyas N, Villapun VM, Cox SC, Jabbari S, Sammons RL, Shelton RM, Walmsley AD, Kuehne SA. Biofilm viability checker: An open-source tool for automated biofilm viability analysis from confocal microscopy images. NPJ Biofilms Microbiomes. 2021 May 14;7(1):44. DOI: 10.1038/s41522-021-00214-7

76. Mandakhalikar KD, Rahmat JN, Chiong E, Neoh KG, Shen L, Tambyah PA. Extraction and quantification of biofilm bacteria: Method optimized for urinary catheters. Sci Rep. 2018 May 23;8(1):8069. DOI: 10.1038/s41598-018-26342-3

77. Schlafer S, Meyer RL. Confocal microscopy imaging of the biofilm matrix. J Microbiol Methods. 2017 Jul;138:50-9. DOI: 10.1016/j.mimet.2016.03.002

78. Celikkol-Aydin S, Gaylarde CC, Lee T, Melchers RE, Witt DL, Beech IB. 16S rRNA gene profiling of planktonic and biofilm microbial populations in the Gulf of Guinea using Illumina NGS. Mar Environ Res. 2016 Dec 1;122:105-12. DOI: 10.1016/j.marenvres.2016.10.001

79. Xu Y, Thomsen TR, Lorenzen J, Chamaon K, Trobisch P, Drange S, editors. Use of next generation sequencing to detect biofilm bacteria in a patient with pedicle screw loosening after spine surgery. EBJIS 2016. 2016 Sep 1.

80. Bouhrour N, Nibbering PH, Bendali F. Medical device-associated biofilm infections and multidrug-resistant pathogens. Pathogens. 2024 May;13(5):393. DOI: 10.3390/pathogens13050393

81. Pantagada N, Kakumanu D, Gowthami P. Biofilm formation and its clinical implications in health care-associated infections. Eur J Cardiovasc Med. 2025 May 8;15:135-40. DOI: 10.5083/ejcm/25-05-26

82. Ben-Amram H, Azrad M, Cohen-Assodi J, Sharabi-Nov A, Edelstein S, Agay-Shay K, Peretz A. Biofilm Formation by Hospital-Acquired Resistant Bacteria Isolated from Respiratory Samples. J Epidemiol Glob Health. 2024 Jun;14(2):291-7. DOI: 10.1007/s44197-024-00215-7

83. Assefa M, Amare A. Biofilm-associated multi-drug resistance in hospital-acquired infections: A review. Infect Drug Resist. 2022;15:5061-8. DOI: 10.2147/IDR.S379502

84. Smith AW. Biofilms and antibiotic therapy: Is there a role for combating bacterial resistance by the use of novel drug delivery systems? Adv Drug Deliv Rev. 2005 Jul 29;57(10):1539-50. DOI: 10.1016/j.addr.2005.04.007

85. de la Fuente-Nunez C, Cesaro A, Hancock REW. Antibiotic failure: Beyond antimicrobial resistance. Drug Resist Updat. 2023 Nov 1;71:101012. DOI: 10.1016/j.drup.2023.101012

86. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001 Jan;9(1):34-9. DOI: 10.1016/s0966-842x(00)01913-2

87. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: An emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019 May 16;8(1):76. DOI: 10.1186/s13756-019-0533-3

88. Hathroubi S, Mekni MA, Domenico P, Nguyen D, Jacques M. Biofilms: Microbial shelters against antibiotics. Microb Drug Resist. 2017 Mar;23(2):147-56. DOI: 10.1089/mdr.2016.0087

89. Sato Y, Unno Y, Ubagai T, Ono Y. Sub-minimum inhibitory concentrations of colistin and polymyxin B promote Acinetobacter baumannii biofilm formation. PLoS ONE. 2018;13(3): e0194556. DOI: 10.1371/journal.pone.0194556

90. Elawady R, Aboulela AG, Gaballah A, Ghazal AA, Amer AN. Antimicrobial sub-MIC induces Staphylococcus aureus biofilm formation without affecting the bacterial count. BMC Infect Dis. 2024 Sep 28;24(1):1065. DOI: 10.1186/s12879-024-09790-3

91. Bal H, Altanlar N, Yildiz S, Bal H, Altanlar N, Yildiz S. The effect of sub-minimal inhibitory concentrations of daptomycin and linezolid on biofilm formation of methicillin resistant staphylococcus aureus isolated from clinical samples. Turk J Pharm Sci. 2025 May 14. DOI: 10.4274/tjps.galenos.2025.26723

92. Veerachamy S, Yarlagadda T, Manivasagam G, Yarlagadda PK. Bacterial adherence and biofilm formation on medical implants: A review. Proc Inst Mech Eng H. 2014 Oct;228(10):1083-99. DOI: 10.1177/0954411914556137

93. Hornschuh M, Zwicker P, Schmidt T, Finke B, Kramer A, Müller G. Poly (hexamethylene biguanide), adsorbed onto Ti-Al-V alloys, kills slime-producing Staphylococci and Pseudomonas aeruginosa without inhibiting SaOs-2 cell differentiation. J Biomed Mater Res B Appl Biomater. 2020 Jul;108(5):1801-13. DOI: 10.1002/jbm.b.34522

94. Hornschuh M, Zwicker P, Schmidt T, Kramer A, Müller G. In vitro evaluation of contact-active antibacterial efficacy of Ti-Al-V alloys coated with the antimicrobial agent PHMB. Acta Biomater. 2020 Apr 1;106:376-86. DOI: 10.1016/j.actbio.2020.02.016

95. Zwicker P, Geist N, Göbler E, Kulke M, Schmidt T, Hornschuh M, Lembke U, Prinz C, Delcea M, Kramer A, Müller G. Improved Adsorption of the antimicrobial agent poly (hexamethylene) biguanide on Ti-Al-V alloys by NaOH treatment and impact of mass coverage and contamination on cytocompatibility. Coatings. 2021;11(9):1118. DOI: 10.3390/coatings11091118

96. Zwicker P, Schmidt T, Hornschuh M, Lode H, Kramer A, Müller G. In vitro response of THP-1 derived macrophages to antimicrobially effective PHMB-coated Ti6Al4V alloy implant material with and without contamination with S. epidermidis and P. aeruginosa. Biomater Res. 2022 Jan 9;26(1):1. DOI: 10.1186/s40824-021-00247-1

97. Müller G, Benkhai H, Matthes R, Finke B, Friedrichs W, Geist N, Langel W, Kramer A. Poly (hexamethylene biguanide) adsorption on hydrogen peroxide treated Ti-Al-V alloys and effects on wettability, antimicrobial efficacy, and cytotoxicity. Biomaterials. 2014 Jul;35(20):5261-77. DOI: 10.1016/j.biomaterials.2014.03.033

98. Makabenta JMV, Nabawy A, Li CH, Schmidt-Malan S, Patel R, Rotello VM. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. Nat Rev Microbiol. 2021 Jan;19(1):23-36. DOI: 10.1038/s41579-020-0420-1

99. Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Deliv Revs. 2013 Nov;65(13-14):1803-15. DOI: 10.1016/j.addr.2013.07.011

100. Mitra D, Kang ET, Neoh KG. Polymer-based coatings with integrated antifouling and bactericidal properties for targeted biomedical applications. ACS Appl Polym Mater. 2021 May 14;3(5):2233-63. DOI: 10.1021/acsapm.1c00125

101. Khan SA, Shakoor A. Recent strategies and future recommendations for the fabrication of antimicrobial, antibiofilm, and antibiofouling biomaterials. Int J Nanomedicine. 2023;18:3377-405. DOI: 10.2147/IJN.S406078

102. Chug MK, Brisbois EJ. Recent developments in multifunctional antimicrobial surfaces and applications toward advanced nitric oxide-based biomaterials. ACS Mater Au. 2022 Sep 14;2(5):525-51. DOI: 10.1021/acsmaterialsau.2c00040

103. Raad I. Intravascular-catheter-related infections. Lancet. 1998 Mar 21;351(9106):893-8. DOI: 10.1016/S0140-6736(97)10006-X

104. Mishra A, Aggarwal A, Khan F. Medical device-associated infections caused by biofilm-forming microbial pathogens and controlling strategies. Antibiotics. 2024 Jul 4;13(7):623. DOI: 10.3390/antibiotics13070623

105. Luchini A, Vitiello G. Understanding the Nano-bio interfaces: Lipid-coatings for inorganic nanoparticles as promising strategy for biomedical applications. Front Chem. 2019;7:343. DOI: 10.3389/fchem.2019.00343

106. Simovic S, Barnes TJ, Tan A, Prestidge CA. Assembling nanoparticle coatings to improve the drug delivery performance of lipid based colloids. Nanoscale. 2012 Feb 21;4(4):1220-30. DOI: 10.1039/c1nr11273b

107. Mashaghi S, Jadidi T, Koenderink G, Mashaghi A. Lipid nanotechnology. Int J Mol Sci. 2013 Feb 21;14(2):4242-82. DOI: 10.3390/ijms14024242

108. Jiménez-Jiménez C, Manzano M, Vallet-Regí M. Nanoparticles coated with cell membranes for biomedical applications. Biol. 2020 Nov 18;9(11):406. DOI: 10.3390/biology9110406

109. Ostadhossein F, Moitra P, Altun E, Dutta D, Sar D, Tripathi I, Hsiao SH, Kravchuk V, Nie S, Pan D. Function-adaptive clustered nanoparticles reverse Streptococcus mutans dental biofilm and maintain microbiota balance. Commun Biol. 2021 Jul 15;4(1):846. DOI: 10.1038/s42003-021-02372-y

110. Nabawy A, Makabenta JM, Li CH, Park J, Chattopadhyay AN, Schmidt-Malan S, Gupta A, Patel R, Rotello VM. Activity of Biodegradable Polymeric Nanosponges against Dual-Species Bacterial Biofilms. ACS Biomater Sci Eng. 2021 May 10;7(5):1780-6. DOI: 10.1021/acsbiomaterials.0c01433

111. Raj V, Kim Y, Kim YG, Lee JH, Lee J. Chitosan-gum arabic embedded alizarin nanocarriers inhibit biofilm formation of multispecies microorganisms. Carbohydr Polym. 2022 May 15;284:118959. DOI: 10.1016/j.carbpol.2021

112. Li X, Chen D, Xie S. Current progress and prospects of organic nanoparticles against bacterial biofilm. Adv Colloid Interface Sci. 2021 Aug;294:102475. DOI: 10.1016/j.cis.2021.102475

113. Uneputty A, Dávila-Lezama A, Garibo D, Oknianska A, Bogdanchikova N, Hernández-Sánchez JF, Susarrey-Arce A. Strategies applied to modify structured and smooth surfaces: A step closer to reduce bacterial adhesion and biofilm formation. Colloid Interface Sci Commun. 2022 Jan 1;46:100560. DOI: 10.1016/j.colcom.2021.100560

114. Yang WJ, Cai T, Neoh KG, Kang ET, Teo SLM, Rittschof D. Barnacle cement as surface anchor for “clicking” of antifouling and antimicrobial polymer brushes on stainless steel. Biomacromolecules. 2013 Jun 10;14(6):2041-51. DOI: 10.1021/bm400382e

115. Zhang B, Yan Q, Yuan S, Zhuang X, Zhang F. Enhanced antifouling and anticorrosion properties of stainless steel by biomimetic anchoring PEGDMA-cross-linking polycationic brushes. Industr Engin Chem Res. 2019 Apr 9;58(17):7107-19. DOI: 10.1021/acs.iecr.8b05599

116. Xu G, Liu P, Pranantyo D, Xu L, Neoh KG, Kang ET. Antifouling and antimicrobial coatings from zwitterionic and cationic binary polymer brushes assembled via “click” reactions. Industr Engin Chem Res. 2017 Dec 4;56(49):14479-88. DOI: 10.1021/acs.iecr.7b03132

117. Wang M, Zheng Y, Yin C, Dai S, Fan X, Jiang Y, Liu X, Fang J, Yi B, Zhou Q, Wang T. Recent progress in antibacterial hydrogel coatings for targeting biofilm to prevent orthopedic implant-associated infections. Front Microbiol. 2023;14:1343202. DOI: 10.3389/fmicb.2023.1343202

118. Al Bataineh MT, Alazzam A. Transforming medical device biofilm control with surface treatment using microfabrication techniques. PloS One. 2023;18(11):e0292647. DOI: 10.1371/journal.pone.0292647

119. Wang N, Ma Y, Shi H, Song Y, Guo S, Yang S. Mg-, Zn-, and Fe-Based Alloys With Antibacterial Properties as Orthopedic Implant Materials. Front Bioeng Biotechnol. 2022 Dec 22;10:888084. DOI: 10.3389/fbioe.2022.888084

120. Barnes I, Cooper I, editors. Biomaterials and Medical Device-associated Infections. Elsevier/Woodhead Publ;2015. Available from: https://books.google.co.in/books?hl=en&lr=&id=QpQAwAAQBAJ&oi=fnd&pg=PP1&dq=Barnes+L

121. Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018 Dec 28;4(12):e01067. DOI: 10.1016/j.heliyon.2018.e01067

122. Vikram A, Jesudhasan PR, Jayaprakasha GK, Pillai SD, Patil BS. Citrus limonoids interfere with Vibrio harveyi cell-cell signalling and biofilm formation by modulating the response regulator LuxO. Microbiol Read Engl. 2011 Jan;157(Pt 1):99-110. DOI: 10.1099/mic.0.041228-0

123. Vikram A, Jayaprakasha GK, Jesudhasan PR, illai SD, Patil BS. Suppression of bacterial cell-cell signalling, biofilm formation and type III secretion system by citrus flavonoids. J Appl Microbiol.2010 Aug;109(2):515-27. DOI: 10.1111/j.1365-2672.2010.04677.x

124. Majtan J, Bohova J, Horniackova M, Klaudiny J, Majtan V. Anti-biofilm effects of honey against wound pathogens Proteus mirabilis and Enterobacter cloacae. Phytother Res. 2014;28(1):69-75. DOI: 10.1002/ptr.4957

125. Bodet C, Piché M, Chandad F, Grenier D. Inhibition of periodontopathogen-derived proteolytic enzymes by a high-molecular-weight fraction isolated from cranberry. J Antimicrob Chemother. 2006 Apr 1;57(4):685-90. DOI: 10.1093/jac/dkl031

126. Gadar K, McCarthy RR. Using next generation antimicrobials to target the mechanisms of infection. Npj Antimicrob Resist. 2023 Sep 22;1(1):1-14. DOI: 10.1038/s44259-023-00011-6

127. Sharma K, Pagedar Singh A. Antibiofilm effect of DNase against Single and mixed species biofilm. Foods. 2018 Mar 19;7(3):42. DOI: 10.3390/foods7030042

128. Kumar Shukla S, Rao TS. Dispersal of Bap-mediated Staphylococcus aureus biofilm by proteinase K. J Antibiot (Tokyo). 2013 Feb;66(2):55-60. DOI: 10.1038/ja.2012.98

129. Fredheim EGA, Klingenberg C, Rohde H, Frankenberger S, Gaustad P, Flægstad T, Ericson Sollid J. Biofilm formation by Staphylococcus haemolyticus. J Clin Microbiol. 2009 Apr;47(4):1172-80. DOI: 10.1128/JCM.01891-08

130. Nguyen UT, Burrows LL. DNase I and proteinase K impair Listeria monocytogenes biofilm formation and induce dispersal of pre-existing biofilms. Int J Food Microbiol. 2014 Sep 18;187:26-32. DOI: 10.1016/j.ijfoodmicro.2014.06.025

131. Svensson A, Larsson A, Emtenäs H, Hedenström M, Fex T, Hultgren SJ, Pinkner JS, Almqvist F, Kihlberg J. Design and evaluation of pilicides: potential novel antibacterial agents directed against uropathogenic Escherichia coli. Chembiochem. 2001 Dec 3;2(12):915-8. DOI: 10.1002/1439-7633(20011203)2:12<915

132. Elbehiry A, Marzouk E, Edrees HM, Ibrahem M, Alzahrani S, Anagreyyah S, Abualola H, Alghamdi A, Alzahrani A, Jaber M, Abu-Okail A. Understanding Pseudomonas aeruginosa biofilms: Quorum sensing, c-di-GMP signaling, and emerging antibiofilm approaches. Microorganisms. 2026 Jan 4;14(1):109. DOI: 10.3390/microorganisms14010109

133. Berne C, Ducret A, Hardy GG, Brun YV. Adhesins involved in attachment to abiotic surfaces by Gram-negative bacteria. Microbiol Spectr. 2015 Aug;3(4):10.1128/microbiolspec.MB-0018-2015. DOI: 10.1128/microbiolspec.MB-0018-2015

134. Izano EA, Amarante MA, Kher WB, Kaplan JB. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms. Appl Environ Microbiol. 2008 Jan;74(2):470-6. DOI: 10.1128/AEM.02073-07

135. Kim HS, Park HD. Ginger extract inhibits biofilm formation by Pseudomonas aeruginosa PA14. PLoS ONE. 2013 Sep 27;8(9):e76106. DOI: 10.1371/journal.pone.0076106

136. Almeida FA de, Vargas ELG, Carneiro DG, Pinto UM, Vanetti MCD. Virtual screening of plant compounds and nonsteroidal anti-inflammatory drugs for inhibition of quorum sensing and biofilm formation in Salmonella. Microb Pathog. 2018 Aug 1;121:369-88. DOI: 10.1016/j.micpath.2018.05.014

137. Zhou JW, Chen TT, Tan XJ, Sheng JY, Jia AQ. Can the quorum sensing inhibitor resveratrol function as an aminoglycoside antibiotic accelerant against Pseudomonas aeruginosa? Int J Antimicrob Agents. 2018 Jul 1;52(1):35-41. DOI: 10.1016/j.ijantimicag.2018.03.002

138. Jia R, Yang D, Xu D, Gu T. Mitigation of a nitrate reducing Pseudomonas aeruginosa biofilm and anaerobic biocorrosion using ciprofloxacin enhanced by D-tyrosine. Sci Rep. 2017 Jul 31;7(1):6946. DOI: 10.1038/s41598-017-07312-7

139. Xu D, Jia R, Li Y, Gu T. Advances in the treatment of problematic industrial biofilms. World J Microbiol Biotechnol. 2017 May;33(5):97. DOI: 10.1007/s11274-016-2203-4

140. Hrynyshyn A, Simões M, Borges A. Biofilms in surgical site infections: Recent advances and novel prevention and eradication strategies. Antibiotics. 2022 Jan 7;11(1):69. DOI: 10.3390/antibiotics11010069

141. Farah H, Kadhim-Abosaoda M, Mohaisen-Mousa H, Renuka Jyothi S, Priyadarshini-Nayak P, Bethanney Janney J, Singh G, Singh-Chauhan A, Kumar-Mishra M. Nanomedicine strategies against biofilm-associated infections: Advances, challenges, and translational barriers. MicrobiologyOpen. 2025 Dec 28;15(1):e70210. DOI: 10.1002/mbo3.70210

142. Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. Biofilms in the food industry: Health aspects and control methods. Front. Microbiol. 2018 May 7;9:898. DOI: 10.3389/fmicb.2018.00898

143. Van Impe J, Smet C, Tiwari B, Greiner R, Ojha S, Stulić V, Vukušić T, Režek Jambrak A. State of the art of nonthermal and thermal processing for inactivation of micro-organisms. J Appl Microbiol. 2018 Jul;125(1):16-35. DOI: 10.1111/jam.13751

144. Yi X, Wang C, Yu X, Su W, Yuan Z. Chitosan/zinc nitrate microneedles for bacterial biofilm eradication. J Biomed Mater Res. 2021;109:911-20. DOI: 10.1002/jbm.b.34755

145. Batoni G, Maisetta G, Esin S. Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. Biochim Biophys Acta. 2016 May 1;1858(5):1044-60. DOI: 10.1016/j.bbamem.2015.10.013

146. Yoon JW, Kang SS. In vitro antibiofilm and anti-inflammatory properties of bacteriocins produced by Pediococcus acidilactici against Enterococcus faecalis. Foodborne Pathog Dis. 2020 Dec;17(12):764-71. DOI: 10.1089/fpd.2020.2804

147. Suryaletha K, Savithri AV, Nayar SA, Asokan S, Rajeswary D, Thomas S. Demystifying bacteriocins of human microbiota by genome guided prospects: An impetus to rekindle the antimicrobial research. Curr Protein Pept Sci. 2022;23(12):811-22. DOI: 10.2174/1389203724666221019111515

148. Sharma G, Dang S, Gupta S, Gabrani R. Antibacterial activity, cytotoxicity, and the mechanism of action of bacteriocin from Bacillus subtilis GAS101. Med Princ Pract. 2018 May;27(2):186-92. DOI: 10.1159/000487306

149. Dutta B, Basu D, Lahiri D, Nag M, Ray RR. Antibacterial and antibiofilm activities of bacteriocin produced by a new strain of Enterococcus faecalis BDR22. Naunyn Schmiedebergs Arch Pharmacol. 2025 Mar;398(3):2983-99. DOI: 10.1007/s00210-024-03458-0

150. Kranjec C, Kristensen SS, Bartkiewicz KT, Brønner M, Cavanagh JP, Srikantam A, Mathiesen G, Diep DB. A bacteriocin-based treatment option for Staphylococcus haemolyticus biofilms. Sci Rep. 2021 Jul 6;11(1):13909. DOI: 10.1038/s41598-021-93158-z

151. Kathju S, Nistico L, Hall-Stoodley L, Post JC, Ehrlich GD, Stoodley P. Chronic surgical site infection due to suture-associated polymicrobial biofilm. Surg Infect. 2009 Oct;10(5):457-61. DOI: 10.1089/sur.2008.062

152. Edmiston CE, McBain AJ, Kiernan M, Leaper DJ. A narrative review of microbial biofilm in postoperative surgical site infections: Clinical presentation and treatment. J Wound Care. 2016 Dec 2;25(12):693-702. DOI: 10.12968/jowc.2016.25.12.693

153. Mihai MM, Preda M, Lungu I, Gestal MC, Popa MI, Holban AM. Nanocoatings for Chronic Wound Repair-Modulation of Microbial Colonization and Biofilm Formation. Int J Mol Sci. 2018 Apr 12;19(4):1179. DOI: 10.3390/ijms19041179

154. Yazici A, Ortucu S, Taskin M, Marinelli L. Natural-based antibiofilm and antimicrobial peptides from microorganisms. Curr Top Med Chem. 2018;18(24):2102-7. DOI: 10.2174/1568026618666181112143351

155. Wuersching SN, Huth KC, Hickel R, Kollmuss M. Targeting antibiotic tolerance in anaerobic biofilms associated with oral diseases: Human antimicrobial peptides LL-37 and lactoferricin enhance the antibiotic efficacy of amoxicillin, clindamycin and metronidazole. Anaerobe. 2021 Oct 1;71:102439. DOI: 10.1016/j.anaerobe.2021.102439

156. Wuersching SN, Huth KC, Hickel R, Kollmuss M. Inhibitory effect of LL-37 and human lactoferricin on growth and biofilm formation of anaerobes associated with oral diseases. Anaerobe. 2021 Feb;67:102301. DOI: 10.1016/j.anaerobe.2020.102301

157. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. Science. 2002 Feb 22;295(5559):1487. DOI: 10.1126/science.295.5559.1487

158. Pourhajibagher M, Etemad-Moghadam S, Alaeddini M, Mousavi RSM, Bahador A. DNA-aptamer-nanographene oxide as a targeted bio-theragnostic system in antimicrobial photodynamic therapy against Porphyromonas gingivalis. Sci Rep. 2022 Jul 16;12(1):12161. DOI: 10.1038/s41598-022-16310-3

159. Gray JA, Chandry PS, Kaur M, Kocharunchitt C, Bowman JP, Fox EM. Novel biocontrol methods for listeria monocytogenes biofilms in food production facilities. Front Microbiol. 2018 Apr 3;9:605. DOI: 10.3389/fmicb.2018.00605

160. Sharma U, Vipra A, Channabasappa S. Phage-derived lysins as potential agents for eradicating biofilms and persisters. Drug Discov Today. 2018 Apr;23(4):848-56. DOI: 10.1016/j.drudis.2018.01.026

161. Cornelissen A, Ceyssens PJ, T'Syen J, Van Praet H, Noben JP, Shaburova OV, Krylov VN, Volckaert G, Lavigne R. The T7-related Pseudomonas putida phage φ15 displays virion-associated biofilm degradation properties. PLoS One. 2011 Apr 19;6(4):e18597. DOI: 10.1371/journal.pone.0018597

162. Sybesma W, Zbinden R, Chanishvili N, Kutateladze M, Chkhotua A, Ujmajuridze A, Mehnert U, Kessler TM. Bacteriophages as Potential Treatment for Urinary Tract Infections. Front Microbiol. 2016 Apr 11;7:465. DOI: 10.3389/fmicb.2016.00465

163. Hemmati F, Rezaee MA, Ebrahimzadeh S, Yousefi L, Nouri R, Kafil HS, Gholizadeh P. Novel Strategies to Combat Bacterial Biofilms. Mol Biotechnol. 2021 Jul;63(7):569-86. DOI: 10.1007/s12033-021-00325-8

164. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. 2023 Apr 6;13:1137947. DOI: 10.3389/fcimb.2023.1137947

165. Ivanova K, Fernandes MM, Francesko A, Mendoza E, Guezguez J, Burnet M, Tzanov T. Quorum-Quenching and Matrix-Degrading Enzymes in Multilayer Coatings Synergistically Prevent Bacterial Biofilm Formation on Urinary Catheters. ACS Appl Mater Interfaces. 2015 Dec 16;7(49):27066-77. DOI: 10.1021/acsami.5b09489

166. Perwez M, Ahmad R, Sardar M. A reusable multipurpose magnetic nanobiocatalyst for industrial applications. Int J Biol Macromol. 2017 Oct 1;103:16-24. DOI: 10.1016/j.ijbiomac.2017.05.029

167. Perwez M, Mazumder JA, Noori R, Sardar M. Magnetic combi CLEA for inhibition of bacterial biofilm: A green approach. Int J Biol Macromol. 2021 Sep 1;186:780-7. DOI: 10.1016/j.ijbiomac.2021.07.091

168. Loera-Muro A, Guerrero-Barrera A, Tremblay DNY, Hathroubi S, Angulo C. Bacterial biofilm-derived antigens: a new strategy for vaccine development against infectious diseases. Expert Rev Vaccines. 2021 Apr;20(4):385-96. DOI: 10.1080/14760584.2021.1892492

169. Carriquiriborde F, Martin Aispuro P, Ambrosis N, Zurita E, Bottero D, Gaillard ME, Castuma C, Rudi E, Lodeiro A, Hozbor DF. Pertussis Vaccine Candidate Based on Outer Membrane Vesicles Derived From Biofilm Culture. Front Immunol. 2021 Sep 15;12:730434. DOI: 10.3389/fimmu.2021.730434

170. Zurita ME, Wilk MM, Carriquiriborde F, Bartel E, Moreno G, Misiak A, Mills KHG, Hozbor D. A Pertussis Outer Membrane Vesicle-Based Vaccine Induces Lung-Resident Memory CD4 T Cells and Protection Against Bordetella pertussis, Including Pertactin Deficient Strains. Front Cell Infect Microbiol. 2019 Apr 26;9:125. DOI: 10.3389/fcimb.2019.00125

171. Martinez LR, Christaki E, Casadevall A. Specific Antibody to Cryptococcus neoformans glucuronoxylomannan antagonizes antifungal drug action against cryptococcal biofilms In Vitro. J Infect Dis. 2006 Jul 15;194(2):261-6. DOI: 10.1086/504722

172. Martinez LR, Bryan RA, Apostolidis C, Morgenstern A, Casadevall A, Dadachova E. Antibody-guided alpha radiation effectively damages fungal biofilms. Antimicrob Agents Chemother. 2006 Jun;50(6):2132-6. DOI: 10.1128/AAC.00120-06

173. Andes D, Nett J, Oschel P, Albrecht R, Marchillo K, Pitula A. Development and characterization of an in vivo central venous catheter Candida albicans biofilm model. Infect Immun. 2004 Oct;72(10):6023-31. DOI: 10.1128/iai.72.10.6023-6031.2004

174. Martinez LR, Fries BC. Fungal biofilms: Relevance in the setting of human disease. Curr Fungal Infect Rep. 2010 Dec 1;4(4):266-75. DOI: 10.1007/s12281-010-0035-5

175. Tobudic S, Kratzer C, Lassnigg A, Graninger W, Presterl E. In vitro activity of antifungal combinations against Candida albicans biofilms. J Antimicrob Chemother. 2010 Feb 1;65(2):271-4. DOI: 10.1093/jac/dkp429

176. Haddad PA, Mah TF, Mussivand T. In vitro assessment of electric currents increasing the effectiveness of vancomycin against Staphylococcus epidermidis Biofilms. Artif Organs. 2016 Aug;40(8):804-10. DOI: 10.1111/aor.12678

177. Freebairn D, Linton D, Harkin-Jones E, Jones DS, Gilmore BF, Gorman SP. Electrical methods of controlling bacterial adhesion and biofilm on device surfaces. Expert Rev Med Devices. 2013 Jan 1;10(1):85-103. DOI: 10.1586/erd.12.70

178. Del Pozo JL, Rouse MS, Patel R. Bioelectric effect and bacterial biofilms. A systematic review. Int J Artif Organs. 2008 Sep;31(9):786-95. DOI: 10.1177/039139880803100906

179. Kopel M, Degtyar E, Banin E. Surface acoustic waves increase the susceptibility of Pseudomonas aeruginosa biofilms to antibiotic treatment. Biofouling. 2011 Aug;27(7):701-11. DOI: 10.1080/08927014.2011.597051

180. Liu JD, Van Treeck KE, Marston WA, Papadopoulou V, Rowe SE. Ultrasound-mediated antibiotic delivery to in vivo biofilm infections: A review. Chembiochem Eur J Chem Biol. 2024 Oct 16;25(20):e202400181. DOI: 10.1002/cbic.202400181

181. Giannelli M, Landini G, Materassi F, Chellini F, Antonelli A, Tani A, Nosi D, Zecchi-Orlandini S, Rossolini GM, Bani D. Effects of photodynamic laser and violet-blue led irradiation on Staphylococcus aureus biofilm and Escherichia coli lipopolysaccharide attached to moderately rough titanium surface: in vitro study. Lasers Med Sci. 2017 May;32(4):857-64. DOI: 10.1007/s10103-017-2185-y

182. Yin R, Dai T, Avci P, Jorge AE, de Melo WC, Vecchio D, Huang YY, Gupta A, Hamblin MR. Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. Curr Opin Pharmacol. 2013 Oct;13(5):731-62. DOI: 10.1016/j.coph.2013.08.009

183. Hu X, Huang YY, Wang Y, Wang X, Hamblin MR. Antimicrobial photodynamic therapy to control clinically relevant biofilm infections. Front Microbiol. 2018 Jun 27;9:1299. DOI: 10.3389/fmicb.2018.01299

184. Wan F, Draz MS, Gu M, Yu W, Ruan Z, Luo Q. Novel strategy to combat antibiotic resistance: A sight into the combination of CRISPR/Cas9 and nanoparticles. Pharmaceutics. 2021 Mar 8;13(3):352. DOI: 10.3390/pharmaceutics13030352

185. Zhang R, Xu W, Shao S, Wang Q. Gene Silencing Through CRISPR interference in bacteria: Current advances and future prospects. Front Microbiol. 2021 Mar 31;12:635227. DOI: 10.3389/fmicb.2021.635227

186. van Belkum A, Soriaga LB, LaFave MC, Akella S, Veyrieras JB, Barbu EM, Shortridge D, Blanc B, Hannum G, Zambardi G, Miller K, Enright MC, Mugnier N, Brami D, Schicklin S, Felderman M, Schwartz AS, Richardson TH, Peterson TC, Hubby B, Cady KC. Phylogenetic Distribution of CRISPR-Cas Systems in Antibiotic-Resistant Pseudomonas aeruginosa. mBio. 2015 Nov 24;6(6):e01796-15. DOI: 10.1128/mBio.01796-15

187. Palacios Araya D, Palmer KL, Duerkop BA. CRISPR-based antimicrobials to obstruct antibiotic-resistant and pathogenic bacteria. PLoS Pathog. 2021 Jul;17(7):e1009672. DOI: 10.1371/journal.ppat.1009672

188. Li P, Wan P, Zhao R, Chen J, Li X, Li J, Xiong W, Zeng Z. Targeted Elimination of blaNDM-5 Gene in Escherichia coli by Conjugative CRISPR-Cas9 System. Infect Drug Resist. 2022 Apr 8;15:1707-16. DOI: 10.2147/IDR.S357470

189. Saffari Natanzi A, Poudineh M, Karimi E, Khaledi A, Haddad Kashani H. Innovative approaches to combat antibiotic resistance: Integrating CRISPR/Cas9 and nanoparticles against biofilm-driven infections. BMC Med. 2025 Aug 20;23:486. DOI: 10.1186/s12916-025-04323-4

190. Mayorga-Ramos A, Zúñiga-Miranda J, Carrera-Pacheco SE, Barba-Ostria C, Guamán LP. CRISPR-Cas-based antimicrobials: Design, challenges, and bacterial mechanisms of resistance. ACS Infect Dis. 2023 Jul 14;9(7):1283-302. DOI: 10.1021/acsinfecdis.2c00649

191. Shatila F, Yaşa İ, Yalçın HT. Inhibition of Salmonella enteritidis biofilms by Salmonella invasion protein-targeting aptamer. Biotechnol Lett. 2020 Oct;42(10):1963-74. DOI: 10.1007/s10529-020-02920-2

192. Mao B, Cheng L, Wang S, Zhou J, Deng L. Combat biofilm by bacteriostatic aptamer-functionalized graphene oxide. Biotechnol Appl Biochem. 2018;65(3):355-61. DOI: 10.1002/bab.1631

193. Pereira HS, Tagliaferri TL, Mendes TA de O. Enlarging the toolbox against antimicrobial resistance: Aptamers and CRISPR-Cas. Front Microbiol. 2021 Feb 19;12:606360. DOI: 10.3389/fmicb.2021.606360

194. Lee HT, Kim SK, Yoon JW. Antisense peptide nucleic acids as a potential anti-infective agent. J Microbiol. 2019 Jun 1;57(6):423-30. DOI: 10.1007/s12275-019-8635-4

195. Wojciechowska M, Równicki M, Mieczkowski A, Miszkiewicz J, Trylska J. Antibacterial Peptide nucleic acids—facts and perspectives. Molecules. 2020 Jan 28;25(3):559. DOI: 10.3390/molecules25030559

196. Narenji H, Teymournejad O, Rezaee MA, Taghizadeh S, Mehramuz B, Aghazadeh M, Asgharzadeh M, Madhi M, Gholizadeh P, Ganbarov K, Yousefi M, Pakravan A, Dal T, Ahmadi R, Samadi Kafil H. Antisense peptide nucleic acids againstftsZ andefaA genes inhibit growth and biofilm formation of Enterococcus faecalis. Microb Pathog. 2020 Feb 1;139:103907. DOI: 10.1016/j.micpath.2019.103907

197. Otsuka T, Kitami O, Kondo K, Ota H, Oshima S, Tsuchiya A, Shirai T, Fujii K, Nakamure M, Shoji Y, Nakamura H, Masuda Y, Komiyama K, Yoshida K, Ishikawa Y, Iwaya A, Takahashi S, Okazaki M, Hotomi M, Yamanaka N. Incidence survey of acute otitis media in children in Sado Island, Japan–Sado Otitis Media Study (SADOMS). PLoS One. 2013 Jul 2;8(7):e68711. DOI: 10.1371/journal.pone.0068711

198. Castillo JI, Równicki M, Wojciechowska M, Trylska J. Antimicrobial synergy between mRNA targeted peptide nucleic acid and antibiotics in E. coli. Bioorg Med Chem Lett. 2018 Oct 1;28(18):3094-8. DOI: 10.1016/j.bmcl.2018.07.037

199. Brodyagin N, Katkevics M, Kotikam V, Ryan CA, Rozners E. Chemical approaches to discover the full potential of peptide nucleic acids in biomedical applications. Beilstein J Org Chem. 2021;17:1641-88. DOI: 10.3762/bjoc.17.116

200. Ribeiro M, Monteiro FJ, Ferraz MP. Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter. 2012;2(4):176-94. DOI: 10.4161/biom.22905

201. Fux CA, Stoodley P, Hall-Stoodley L, Costerton JW. Bacterial biofilms: a diagnostic and therapeutic challenge. Expert Rev Anti Infect Ther. 2003 Dec;1(4):667-83. DOI: 10.1586/14787210.1.4.667

202. Sadykov MR, Windham IH, Widhelm TJ, Yajjala VK, Watson SM, Endres JL, Bavari AI, Thomas VC, Bose JL, Bayles KW. CidR and CcpA Synergistically Regulate Staphylococcus aureus cidABC Expression. J Bacteriol. 2019 Nov 5;201(23):e00371-19. DOI: 10.1128/JB.00371-19

203. Liu H, Chen H, Sun Y, Zhang X, Lu H, Li J, Cao J, Zhou T. Characterization of the mechanism and impact of staphylokinase on the formation of Candida albicans and Staphylococcus aureus polymicrobial biofilms. J Med Microbiol. 2019 Mar;68(3):355-67. DOI: 10.1099/jmm.0.000914

204. Zheng M, Zhu K, Peng H, Shang W, Zhao Y, Lu S, Rao X, Li M, Zhou R, Li G. CcpA Regulates Staphylococcus aureus Biofilm Formation through Direct Repression of Staphylokinase Expression. Antibiotics (Basel). 2022 Oct 17;11(10):1426. DOI: 10.3390/antibiotics11101426

205. Cegelski L, Pinkner JS, Hammer ND, Cusumano CK, Hung CS, Chorell E, Aberg V, Walker JN, Seed PC, Almqvist F, Chapman MR, Hultgren SJ. Small-molecule inhibitors target Escherichia coli amyloid biogenesis and biofilm formation. Nat Chem Biol. 2009 Dec;5(12):913-9. DOI: 10.1038/nchembio.242

206. Romero D, Sanabria-Valentín E, Vlamakis H, Kolter R. Biofilm inhibitors that target amyloid proteins. Chem Biol. 2013 Jan 24;20(1):102-10. DOI: 10.1016/j.chembiol.2012.10.021

207. Hernández-Huerta MT, Pérez-Campos E, Pérez-Campos Mayoral L, Vásquez Martínez IP, Reyna González W, Jarquín González EE, Aldossary H, Alhabib I, Yamani LZ, Elhadi N, Al-Suhaimi E, Cabrera-Fuentes HA. Proactive Strategies to Prevent Biofilm-Associated Infections: From Mechanistic Insights to Clinical Translation. Microorganisms. 2025 Dec;13(12):2726. DOI: 10.3390/microorganisms13122726

208. Grari O, Ezrari S, El Yandouzi I, Benaissa E, Ben Lahlou Y, Lahmer M, Saddari A, Elouennass M, Maleb A. A comprehensive review on biofilm-associated infections: Mechanisms, diagnostic challenges, and innovative therapeutic strategies. Microbe. 2025 Sep 1;8:100436. DOI: 10.1016/j.microb.2025.100436

209. Xie Y, Liu H, Teng Z, Ma J, Liu G. Nanomaterial-enabled anti-biofilm strategies: New opportunities for treatment of bacterial infections. Nanoscale. 2025 Feb;17(10):5605-28. DOI: 10.1039/D4NR04774E

210. Venkataiah VS, Karobari MI. Effectiveness of biofilm-targeted therapy in managing and preventing dental caries: protocol for a systematic review and meta-analysis. Syst Rev. 2025 Aug 11;14(1):165. DOI: 10.1186/s13643-025-02921-0

211. Highmore CJ, Melaugh G, Morris RJ, Parker J, Direito SOL, Romero M, Soukarieh F, Robertson SN, Bamford NC. Translational challenges and opportunities in biofilm science: a BRIEF for the future. NPJ Biofilms Microbiomes. 2022 Aug 29;8(1):68. DOI: 10.1038/s41522-022-00327-7

212. Robertson SN, Romero M, Fenn S, Kohler Riedi PL, Cámara M. Development, characterization, and evaluation of a simple polymicrobial colony biofilm model for testing of antimicrobial wound dressings. J Appl Microbiol. 2024 Mar 1;135(3):lxae042. DOI: 10.1093/jambio/lxae042

213. Fisher RA, Gollan B, Helaine S. Persistent bacterial infections and persister cells. Nat Rev Microbiol. 2017 Aug;15(8):453-64. DOI: 10.1038/nrmicro.2017.42

214. Hall-Stoodley L, Stoodley P, Kathju S, Høiby N, Moser C, Costerton JW, Moter A, Bjarnsholt T. Towards diagnostic guidelines for biofilm-associated infections. FEMS Immunol Med Microbiol. 2012 Jul;65(2):127-45. DOI: 10.1111/j.1574-695X.2012.00968.x

215. Liu Y, Zhang J, Ji Y. PCR-based approaches for the detection of clinical methicillin-resistant Staphylococcus aureus. Open Microbiol J. 2016 Apr 14;10:45-56. DOI: 10.2174/1874285801610010045

216. Luteijn JM, Hubben GA, Pechlivanoglou P, Bonten MJ, Postma MJ. Diagnostic accuracy of culture-based and PCR-based detection tests for methicillin-resistant Staphylococcus aureus: a meta-analysis. Clin Microbiol Infect. 2011 Feb;17(2):146-54. DOI: 10.1111/j.1469-0691.2010.03202.x

217. Nguyen CQ, Thrift WJ, Bhattacharjee A, Ranjbar S, Gallagher T, Darvishzadeh-Varcheie M, Sanderson RN, Capolino F, Whiteson K, Baldi P, Hochbaum AI, Ragan R. Longitudinal Monitoring of Biofilm Formation via Robust Surface-Enhanced Raman Scattering Quantification of Pseudomonas aeruginosa-Produced Metabolites. ACS Appl Mater Interfaces. 2018 Apr 18;10(15):12364-73. DOI: 10.1021/acsami.7b1859285

218. Kim YW, Meyer MT, Berkovich A, Subramanian S, Iliadis AA, Bentley WE, Ghodssi R. A surface acoustic wave biofilm sensor integrated with a treatment method based on the bioelectric effect. Sens Actuators Phys. 2016;238:140-9. DOI: 10.1016/j.sna.2015.12.001

219. Lora-Tamayo J, Murillo O, Ariza J. Clinical use of colistin in biofilm-associated infections. Adv Exp Med Biol. 2019;1145:181-95. DOI: 10.1007/978-3-030-16373-0_13

220. Kolpen M, Appeldorff CF, Brandt S, Mousavi N, Kragh KN, Aydogan S, Uppal HA, Bjarnsholt T, Ciofu O, Høiby N, Jensen PØ. Increased bactericidal activity of colistin on Pseudomonas aeruginosa biofilms in anaerobic conditions. Pathog Dis. 2016 Feb;74(1):ftv086. DOI: 10.1093/femspd/ftv086

221. Lora-Tamayo J, Murillo O, Bergen PJ, Nation RL, Poudyal A, Luo X, Yu HY, Ariza J, Li J. Activity of colistin combined with doripenem at clinically relevant concentrations against multidrug-resistant Pseudomonas aeruginosa in an in vitro dynamic biofilm model. J Antimicrob Chemother. 2014 Sep;69(9):2434-42. DOI: 10.1093/jac/dku151

222. John AK, Baldoni D, Haschke M, Rentsch K, Schaerli P, Zimmerli W, Trampuz A. Efficacy of daptomycin in implant-associated infection due to methicillin-resistant Staphylococcus aureus: importance of combination with rifampin. Antimicrob Agents Chemother. 2009 Jul;53(7):2719-24. DOI: 10.1128/AAC.00047-09

223. LaPlante KL, Woodmansee S. Activities of daptomycin and vancomycin alone and in combination with rifampin and gentamicin against biofilm-forming methicillin-resistant Staphylococcus aureus isolates in an experimental model of endocarditis. Antimicrob Agents Chemother. 2009 Sep;53(9):3880-6. DOI: 10.1128/AAC.00134-09

224. Nalca Y, Jänsch L, Bredenbruch F, Geffers R, Buer J, Häussler S. Quorum-sensing antagonistic activities of azithromycin in Pseudomonas aeruginosa PAO1: a global approach. Antimicrob Agents Chemother. 2006 May;50(5):1680-8. DOI: 10.1128/AAC.50.5.1680-1688.2006

225. Hoffmann N, Lee B, Hentzer M, Rasmussen TB, Song Z, Johansen HK, Givskov M, Høiby N. Azithromycin blocks quorum sensing and alginate polymer formation and increases the sensitivity to serum and stationary-growth-phase killing of Pseudomonas aeruginosa and attenuates chronic P. aeruginosa lung infection in Cftr(-/-) mice. Antimicrob Agents Chemother. 2007 Oct;51(10):3677-87. DOI: 10.1128/AAC.01011-06

226. Kaplan JB. Therapeutic potential of biofilm-dispersing enzymes. Int J Artif Organs. 2009 Sep;32(9):545-54. DOI: 10.1177/039139880903200903

227. Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun. 2008 Sep;76(9):4176-82. DOI: 10.1128/IAI.00318-08

228. Wardell SJT, Yung DBY, Gupta A, Bostina M, Overhage J, Hancock REW, Pletzer D. DJK-5, an anti-biofilm peptide, increases Staphylococcus aureus sensitivity to colistin killing in co-biofilms with Pseudomonas aeruginosa. NPJ Biofilms Microbiomes. 2025 Jan 8;11(1):8. DOI: 10.1038/s41522-024-00637-y

229. Ahamed M, Alsalhi MS, Siddiqui MKJ. Silver nanoparticle applications and human health. Clin Chim Acta Int J Clin Chem. 2010 Dec 14;411(23-24):1841-8. DOI: 10.1016/j.cca.2010.08.016

230. Onsare JG, Arora DS. Antibiofilm potential of flavonoids extracted from Moringa oleifera seed coat against Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans. J Appl Microbiol. 2015 Feb;118(2):313-25. DOI: 10.1111/jam.12701

231. Liu Y, Zhu J, Liu Z, Zhi Y, Mei C, Wang H. Flavonoids as Promising natural compounds for combating bacterial infections. Int J Mol Sci. 2025 Jan;26(6):2455. DOI: 10.3390/ijms26062455

232. Bartolomeu M, Rocha S, Cunha Â, Neves MGPMS, Faustino MAF, Almeida A. Effect of photodynamic therapy on the virulence factors of Staphylococcus aureus. Front Microbiol. 2016 March 7;7:267. DOI: 10.3389/fmicb.2016.00267

233. Huang YY, Tanaka M, Vecchio D, Garcia-Diaz M, Chang J, Morimoto Y, Hamblin MR. Photodynamic therapy induces an immune response against a bacterial pathogen. Expert Rev Clin Immunol. 2012 Jul;8(5):479-94. DOI: 10.1586/eci.12.37

234. Rupp ME, Fitzgerald T, Marion N, Helget V, Puumala S, Anderson JR, Fey PD. Effect of silver-coated urinary catheters: efficacy, cost-effectiveness, and antimicrobial resistance. Am J Infect Control. 2004 Dec;32(8):445-50. DOI: 10.1016/j.ajic.2004.05.002

235. Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008 Jan;21(1):26-59. DOI: 10.1128/CMR.00019-07

236. Shein AMS, Wannigama DL, Higgins PG, Hurst C, Abe S, Hongsing P, Chantaravisoot N, Saethang T, Luk-In S, Liao T, Nilgate S, Rirerm U, Kueakulpattana N, Laowansiri M, Srisakul S, Muhummudaree N, Techawiwattanaboon T, Gan L, Xu C, Kupwiwat R, Phattharapornjaroen P, Rojanathanes R, Leelahavanichkul A, Chatsuwan T. Novel colistin-EDTA combination for successful eradication of colistin-resistant Klebsiella pneumoniae catheter-related biofilm infections. Sci Rep. 2021 Nov 4;11(1): 21676). DOI: 10.1038/s41598-021-01052-5

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