CRITICAL REVIEW

WHS Wound Healing Society

Overcoming Challenges in Wound Infection: Biofilm and Antimicrobial Resistance

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Significance: Chronic wounds represent a growing clinical and economic burden, affecting 1–2% of the global population, with prevalence expected to rise due to aging and increasing rates of diabetes, obesity, and vascular diseases. Wound persistence is often driven by infection and compounded by antimicrobial resistance (AMR), resulting in poor patient outcomes. High prevalence of microbial biofilms, which shield pathogens from immune clearance and promote AMR, further promotes the chronicity of infected wounds.
Recent Advances: Adoption of antimicrobial stewardship in wound care is increasing, emphasizing timely diagnosis of infection and pathogen identification to guide treatment and limit unnecessary AMR-driving antibiotic use. However, current diagnostic and therapeutic approaches remain only partially effective, particularly for biofilm-containing wounds. While many antimicrobials exist, their use is constrained by negative impacts on wound healing, limited antibiofilm activity, and insufficient evidence of improved clinical outcome. To address these gaps, recent advances in diagnostics and therapeutics aim to disrupt microbial communities, reduce AMR risk, and accelerate wound healing.
Critical Issues: The treatment of chronic wounds is challenged by AMR, biofilms, and the limited effectiveness of current therapies. Contemporary antimicrobials (*e.g.,* broad-spectrum antibiotics and silver) are linked to AMR development, compounded by biofilms that shield pathogens, limit antimicrobial efficacy, and sustain infection. While alternative treatments with lower AMR risk and greater antibiofilm activity are under investigation, the lack of robust clinical data limits their adoption.
Future Directions: Broader adoption of antimicrobial stewardship and biofilm-targeting sustainable wound care practices are key for combatting AMR and improving patient outcomes.

Keywords: antimicrobial resistance, biofilm, chronic wounds, wound infection, wound treatment

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SCOPE AND SIGNIFICANCE

Wound infections represent a major clinical challenge, contributing to increased morbidity, prolonged hospitalization, reduced quality of life, and substantial health care costs.[1] Infection can impact both acute and chronic wounds, presenting significant global health challenges when complicated by biofilms and antimicrobial resistance (AMR) bacteria.[2,3] This review provides a mechanistic

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focus on wound chronicity, biofilms, and AMR (Fig. 1). Specifically, it explores how biofilms contribute to chronic inflammation, immune evasion, and antimicrobial tolerance; outlines mechanisms of AMR; and considers both the diagnostic challenges and therapeutic opportunities in managing infected and chronic wounds, highlighting strategies that target biofilms and reduce AMR risk.

TRANSLATIONAL RELEVANCE

Biofilm-associated infections are notoriously recalcitrant to conventional antimicrobials, necessitating alternative strategies for effective treatment.[4] Such strategies require a nuanced understanding of underlying resistance mechanisms.[5] Extensive research and development, both preclinical and clinical, have led to the emergence of novel wound care strategies that aim to disrupt biofilm integrity, enhance antimicrobial penetration and targeted delivery, restore host immune function, and reduce the risk of AMR development.[6–8] Despite these advances, few products have made it into clinical practice; thus, further research is required to develop novel, cost-effective therapies that promote faster healing, reduce complications, and limit AMR development.

CLINICAL RELEVANCE

Due to persistent diagnostic uncertainty, empirical systemic antibiotics are frequently prescribed for infected and chronic wounds. Although systemic therapy is warranted in some cases, inappropriate or excessive use increases AMR risk and exposes patients to otherwise avoidable side effects.[9] To reduce these complications, antibiotic stewardship programs (ASPs) have become critical for guiding appropriate antibiotic prescribing and have led to fewer hospital-acquired infections and reduced rates of AMR organisms.[10–12] However, despite high awareness, barriers to consistent implementation remain, including limited measurement of the impact of ASPs in wound care and insufficient resources.[13,14] As such, continued incorporation of stewardship principles into the design of new wound care products and therapies will be essential for reducing AMR- and biofilm-driven infection persistence.

BACKGROUND

Chronic wounds, defined as those that fail to progress through the normal stages of healing in a timely manner, affect 1–2% of the global population.[15] Their prevalence is expected to rise with aging populations and increasing rates of diabetes, obesity, metabolic disorders, and vascular

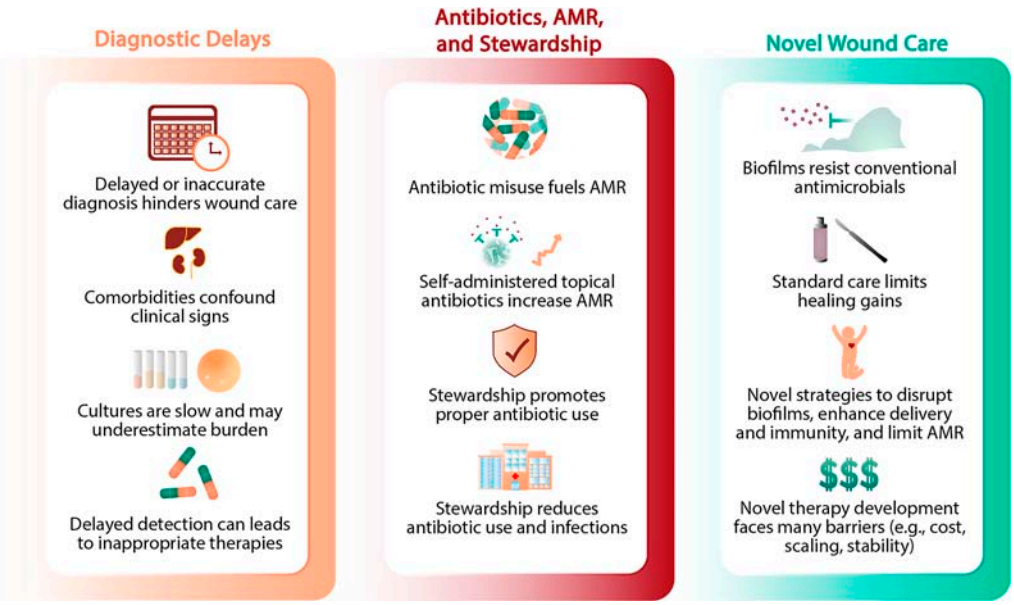

Figure 1. Summary graphic. Delayed or inaccurate diagnosis of wound infection hinders optimal care and contributes to antimicrobial resistance (AMR). Accurate diagnosis is challenged by confounding clinical signs from comorbidities and the limitations of traditional microbial cultures, which are slow and often underestimate the diversity and extent of microbial burden, especially when a biofilm is present. Delays in accurate detection can lead to inappropriate or less effective therapies, while misuse of systemic and topical antibiotics fuels AMR. These challenges underscore the need for antimicrobial stewardship programs promoting judicious antibiotic use. Given that biofilm-associated infections resist conventional antimicrobial therapies, novel antibiofilm strategies are needed to help limit AMR. However, clinical translation of promising new therapies remains constrained by cost, scalability, stability, required expertise, potential toxicity, and limited evidence for superiority over current standards of care.

complications, all major risk factors for impaired healing.[2,16–18] Diabetic foot ulcers (DFUs) are of particular concern, affecting 18.6 million people worldwide and frequently leading to amputations and high mortality.[19]

The pathogenesis of chronic wound infection involves a dynamic interplay between microbial colonization, host immune responses, and the local wound environment.[20] This complexity is amplified by the frequent development of biofilms, structured communities of bacteria and fungi embedded in self-secreted extracellular polymeric substance (EPS) matrices, that adhere to the wound bed and protect pathogens from host immune defenses and systemic antimicrobials.[21] Biofilm formation is a defining feature of the transition from acute to chronic wounds, driving persistent infection and impaired healing[22–24] in an estimated 70% of chronic cases and underscoring the difficulty of durable healing.[24] Individuals with metabolic dysfunction are at particular risk as chronic inflammation alters the barrier function of the skin and its microbiome (dysbiosis),[25] leading to a higher proportion of pathogenic microbes (*e.g., Staphylococcus aureus* and *Pseudomonas aeruginosa*) and a high incidence of biofilm infection.[26]

Delayed or inaccurate diagnosis of wound infection remains a major barrier to optimal care. Clinical signs can be confounded by underlying comorbidities (*e.g.,* reduced pain sensation in diabetic neuropathy), while standard wound swab cultures are slow and may underestimate microbial diversity, especially when biofilms are present.[27] Failure to rapidly and accurately detect wound infection leads to inappropriate antimicrobial use, AMR, and poor patient outcomes. Current standards of care include physical debridement and topical antiseptics, but these interventions are often insufficient, as biofilms rapidly reform.[28] Topical antibiotics present a parallel concern, as evidence supporting their efficacy in reducing wound infection is limited, while their indiscriminate use (*e.g.,* through patient self-administration) fosters the spread of multidrug-resistant organisms (MDROs) and increases AMR risk.[29] These challenges are magnified in biofilm-containing wounds, where pathogens are shielded from antimicrobials and immune clearance, further driving treatment failures.

To address these challenges, researchers are developing novel antibiofilm strategies that balance efficacy with lower risk of resistance. Traditional antimicrobials, such as antibiotics, iodine, and silver-based compounds, offer broad-spectrum activity but contribute to AMR.[30–32] Alternative approaches, including antiseptics, antimicrobial peptides (AMPs), nanomaterials, and a variety of advanced antimicrobial therapies, aim to mitigate resistance while managing biofilms, infection, and healing of chronic wounds.[10] Although the number of novel approaches continues to grow, significant barriers hinder their translation into clinical practice, which includes manufacturing costs, challenges with scale-up and storage stability, the need for specialized staff training, potential toxicity, and a lack of robust clinical trial evidence.[33] However, as resistance outpaces new therapeutic development, the need for effective treatments has become increasingly urgent, thus necessitating continued research and clinical testing to bring new therapeutic options into the clinic.

DISCUSSION

Clinical wound infection and wound chronicity

Skin wounds typically undergo a well-orchestrated healing process, through which transient microbial contamination by commensal skin flora, environmental microbes, or nosocomial pathogens is effectively cleared by innate immune mechanisms. In healthy individuals, these wounds generally resolve in 2–4 weeks.[21,34] The shift from transient colonization to overt infection occurs when microbial burden overwhelms host defenses or virulent organisms (*e.g., S. aureus*) exploit host vulnerabilities (**Fig. 2**).[35] Infection and inflammation may then reinforce one another, creating a cycle that stalls healing ("wound chronicity"), prolongs treatment, and worsens patient outcomes.[36]

Chronic wounds, defined as those that fail to heal within 4–12 weeks depending on wound type and context,[1,37] arise from impaired healing mechanisms, repeated exposure to environmental pathogens, and persistent inflammation. They are strongly associated with comorbidities, such as autoimmune, metabolic, and cardiovascular diseases, which compromise immune function, perfusion, and oxygen delivery to the wound site. Chronic wounds are more susceptible to infection, which, in turn, reinforces the cycle of wound chronicity.[38] Trauma, burns, and surgical wounds can also increase infection susceptibility and delay wound healing.[36]

The prevalence of chronic wounds increases with age, with pressure ulcers affecting up to 19% of patients in Swiss nursing homes,[39] DFUs occurring in 8% of U.S. Medicare recipients (15% in those over 95 years), and DFU infection rates ranging from 40% to 80% depending on the specific health care setting and diagnostic criteria.[40]

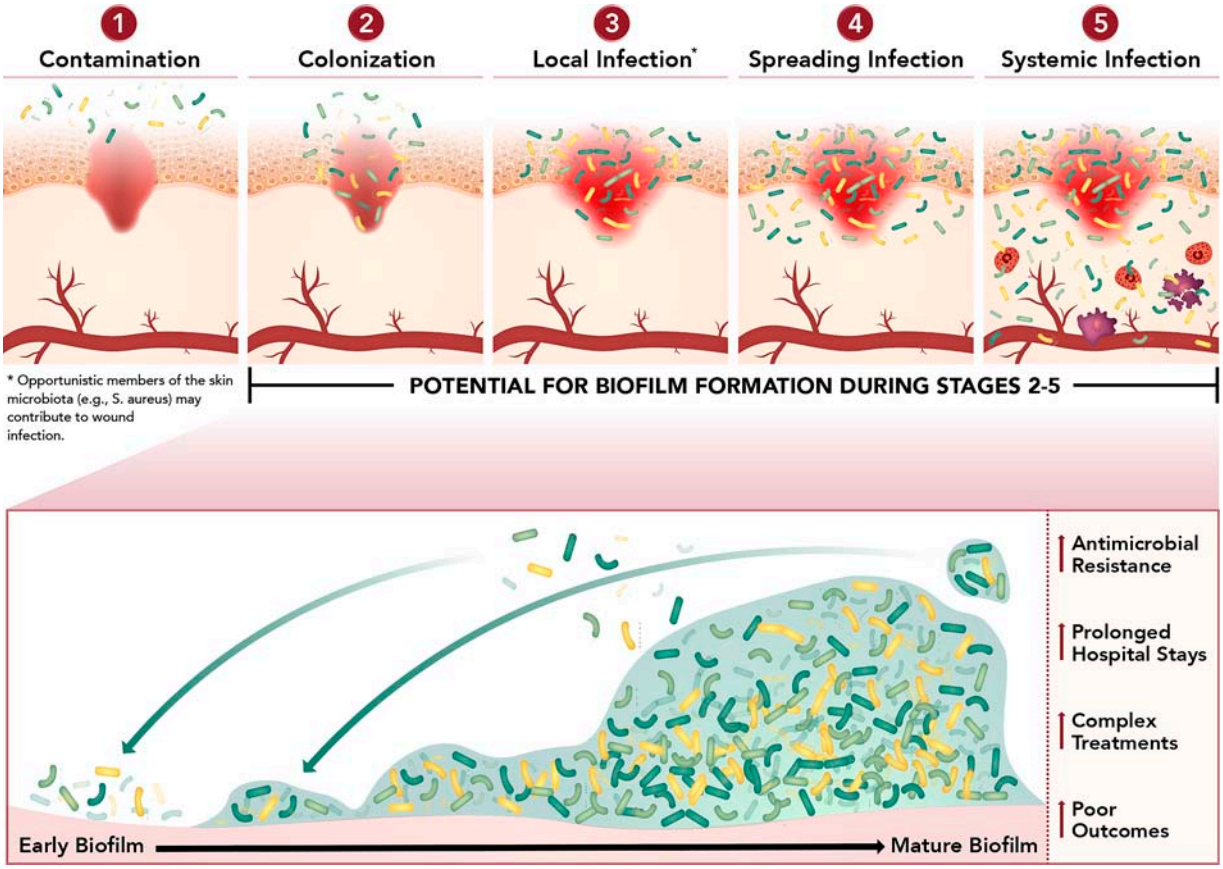

Figure 2. Progression of infections and potential for biofilm formation. The transition from wound colonization to infection is governed by both microbial and host factors. All wounds are initially contaminated by microbes (Stage 1: contamination), but effective immune function prevents proliferation. If the host immune defenses are overwhelmed, low levels of microbial proliferation can ensue (Stage 2: colonization) and ultimately progress to localized infection (Stage 3), characterized by hypergranulation, erythema, and delayed healing. If host defenses remain inadequate, microbial proliferation will continue and the infection will spread (Stage 4: spreading infection). The most severe stage (Stage 5: systemic infection) involves microbial dissemination into the bloodstream, resulting in sepsis, and carries high morbidity and mortality risks. Biofilms can form during any proliferative phase (Stages 2–5), especially under conditions that favor the growth of certain pathogenic microbial strains (*e.g., Staphylococcus aureus*) contributing to chronicity and AMR through their tolerance to standard antimicrobial therapies. Wounds infected with biofilms often require more complex treatments, prolonged hospitalization, and are associated with poor patient outcomes.

Venous leg ulcers are less prevalent but affect 1.5% of individuals over 65 years and are more common in women.[26]

Host defenses

Skin provides both a physical barrier and an active immunological environment that integrates innate and adaptive immunity, AMPs, and the resident microbiome to prevent infection. At sites of injury, neutrophils and macrophages of the innate immune response rapidly initiate local inflammation and microbial clearance through the production and release of reactive oxygen species (ROS) and AMPs (*e.g.,* LL-37). These responses not only kill pathogens but also regulate cytokine production, shape macrophage polarization, and bridge innate and adaptive immunity by activating dendritic cells, promoting antigen presentation, and stimulating T- and B-cell responses.[41] The skin microbiome further contributes, with commensal bacteria (*e.g., Staphylococcus epidermidis*) inhibiting pathogenic growth through competitive exclusion and antimicrobial metabolite production, directly supporting wound healing.[42]

In chronic wounds, these defenses are often disrupted, leading to persistent infection, delayed repair, and greater morbidity. Hypoxia and necrosis within the chronic wound environment impair immune cell recruitment and function, reduce AMP production, dysregulate cytokine signaling, and consequently inhibit effective clearance of pathogens.[41] Chronic inflammation leads to excessive release of inflammatory mediators (*e.g.,* tumor necrosis factor-α, interleukin-1, and interferon gamma) and proteolytic enzymes (*e.g.,* matrix metalloproteases) that degrade growth factors and the extracellular matrix (ECM), preventing the inflammatory (M1) to reparative (M2) macrophage shift

required for tissue healing.[36] Meanwhile, the microbiome shifts toward pathogen dominance, with pathogens *S. aureus* and *P. aeruginosa* secreting virulence factors that facilitate adhesion, invasion, and potential biofilm formation within the wound. Biofilms may then block immune cell penetration, perpetuating immune evasion, inflammation, and wound chronicity,[24,43] and promoting AMR emergence and persistence.[1]

Mechanisms of biofilms in chronic wounds

Chronic wounds provide an ideal environment for biofilm formation, as suggested by the presence of these structured communities in 60–90% of cases compared with only 6% in acute wounds.[1,24,44–49] Once established, they function not as passive colonizers but as dynamic, adaptive ecosystems that fundamentally alter wound pathophysiology. Biofilms delay granulation tissue formation and re-epithelialization, and up to 39% of affected wounds recur within 12 weeks of apparent closure.[50–52] Their resilience reflects a convergence of protective strategies (*e.g.,* physical shielding, metabolic rewiring, immune evasion, polymicrobial cooperation, and genetic diversification) that make them central drivers of wound chronicity, antimicrobial tolerance, AMR, and treatment failure.[23,44–46,48,53–55]

The biofilm developmental cycle is classically described in five stages: initial/reversible adhesion, irreversible adhesion, microcolony formation, maturation (phases I and II), and dispersal (Fig. 3).[44,56] In this model, planktonic bacteria first attach weakly to surfaces, then establish permanent anchoring through adhesins and pili, proliferate into microcolonies, and mature into structured communities with towers, ridges, and channels that optimize nutrient and waste exchange.[44,45,56] Dispersal, triggered by environmental stressors (*e.g.,* nutrient depletion, oxygen shifts, or antimicrobial exposure), releases subsets of bacteria back into the planktonic state, often in a heightened virulence phenotype primed for recolonization.[44,45,56] Importantly, this early model provides a useful framework but does not fully capture the variability observed across different microbial species and environmental conditions.

While surface-associated biofilms are well described in the context of dental plaque or implant-related infections, chronic wound infections also often contain small microbial aggregates suspended in exudate or embedded in necrotic tissue, ranging from 2 to 200 μm in diameter.[46–48,55–58] These aggregates display hallmark biofilm features (*e.g.,* EPS encapsulation, antimicrobial tolerance, AMR, and immune evasion) even without forming the large mushroom-shaped structures typical *in vitro*.[48,56,58] Thus, the organization and behavior of chronic wound-associated microbial communities are context-dependent and dictated by microenvironmental cues.[47,49,56]

Biofilm adhesion is facilitated by host-derived proteins, including fibrin, fibronectin, and collagen, which provide binding sites for bacterial adhesins, pili, and flagella.[45,56] Once anchored, bacteria secrete an EPS of polysaccharides, proteins, lipids, and abundant extracellular deoxyribonucleic acid (eDNA).[1,44,45] The EPS provides structural integrity, reinforces bacterial attachment, and impedes penetration of immune mediators and antimicrobials (Fig. 3).[23,38,44,45,49,55] Host-derived neutrophil DNA and actin from immune cell lysis may also become incorporated, further densifying the matrix.[1]

Gradients of oxygen, nutrients, and pH emerge within wound biofilms due to bacterial metabolism and host immune activity.[1,46,56–58] Peripheral, metabolically active bacteria consume oxygen rapidly, while infiltrating neutrophils further deplete oxygen, creating hypoxic or anoxic cores.[46,55,58] Here, bacteria shift to anaerobic or fermentative metabolism, rendering them less susceptible to antibiotics that rely on active aerobic metabolism (*e.g., β*-lactams and aminoglycosides).[46,55,58] Hypoxia suppresses ROS production, an antibiotic target, while nutrient limitation reduces proton motive force, impairing aminoglycoside uptake.[58] These adaptations create a physiological tolerance (*i.e.,* survival without growth in response to a given antibiotic) distinct from but often preceding genetic resistance (*i.e.,* growth in the presence of a given antibiotic) and allow survival even at transiently high antibiotic concentrations.[1,48] Consequently, biofilm-associated bacteria are up to 1,000-fold more resistant to antibiotics than their planktonic counterparts.[48,55,59]

These microenvironmental pressures not only drive physiological tolerance but also underpin the spatial and phenotypic heterogeneity characteristic of chronic wound biofilms.[1,44–46,48,55–58] Outer-layer cells remain metabolically active, while inner populations persist in slow-growing or dormant states, creating a division of labor that enhances survival.[55] Persister cells adopt dormant states, withstand antibiotics, and rapidly reseed biofilms posttreatment.[44,53,54] Small colony variants, with altered metabolism and slower growth, survive intracellularly and evade multiple antibiotic classes.[58] Transcriptomic studies of chronic wound

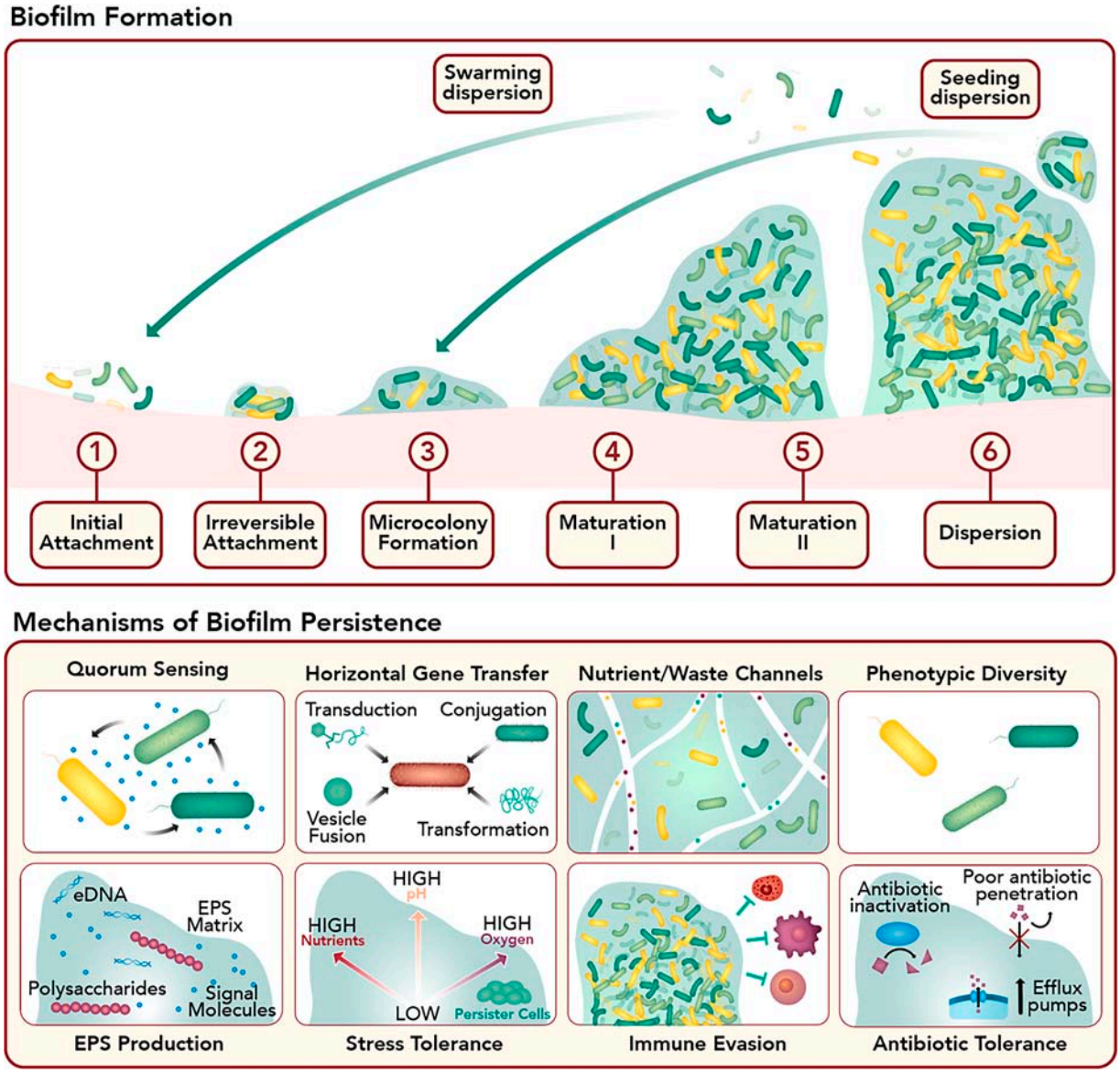

Figure 3. Biofilm formation and mechanisms of persistence and healing perturbation. Biofilm formation proceeds through a series of coordinated stages. During *initial attachment*, planktonic bacteria exhibit weak, reversible adherence to a surface. With adhesin production and secretion of extracellular polymeric substance (EPS), attachment may become irreversible (*irreversible attachment*), and recruited cells begin *microcolony formation*. Biofilm maturation (*maturation I* and *maturation II*) involves increased EPS synthesis, establishment of nutrient and waste transport channels, and formation of structured three-dimensional microbial communities with high cell density and distinct metabolic niches. Once mature, biofilms can disperse, as environmental cues trigger bacterial detachment and colonization of new surfaces through seeding or swarming mechanisms (*dispersion*). Biofilms persist and evade antimicrobial eradication through multiple mechanisms. Quorum sensing enables cell-density–dependent communication that coordinates EPS production and gene expression. Microbes within biofilms engage in horizontal gene transfer, including transduction (through bacteriophages), conjugation (direct cell-to-cell), vesicle fusion, and transformation (DNA uptake), promoting spread of resistance genes. The EPS matrix consisting of DNA, proteins, and polysaccharides supports resource distribution, waste removal, biofilm stability, and tolerance to stressors, such as high pH, nutrient deprivation, or hypoxia. The presence of persister cells and phenotypic heterogeneity further enhances survival under hostile conditions. Biofilm architecture impedes immune cell penetration and phagocytosis, while inactivating enzymes, poor antibiotic penetration, and upregulated efflux pumps collectively antimicrobial efficacy, allowing biofilms to persist despite host immunity and treatment.

isolates further confirm upregulation of efflux pumps, oxidative stress defenses, and virulence determinants, with concurrent downregulation of growth-related genes.[60–62] This diversification ensures survival of at least some subpopulations against any stressor and explains, in part, why chronic wound biofilms are so difficult to eradicate.

Chronic wound biofilms are also frequently polymicrobial, comprising both bacterial and fungal species.[1,44,45,47,58] These organisms are not randomly intermixed but occupy distinct spatial niches.[46,47] While *S. aureus* is frequently more abundant in superficial wound layers, *P. aeruginosa* is more commonly detected within deeper

tissue compartments, a pattern that likely reflects difference in oxygen and nutrient utilization.[44,46,48] Interactions between species further shape biofilm physiology, with cross-feeding sustaining partners through metabolic byproducts and quorum sensing (QS) synchronizing collective behavior.[44] In *P. aeruginosa,* the las and rhl QS systems regulate rhamnolipids, lectins, siderophores, and eDNA, consolidating EPS and suppressing immune clearance[44,46] while upregulating multidrug efflux pumps.[55,63,64] In *S. aureus,* QS governs adhesins and biofilm-associated proteins, coordinating transitions from adhesion to maturation. *Candida albicans* fungi stabilize the matrix and amplify tolerance, while otherwise benign commensals may become pathogenic under stress.[44] Debate remains regarding the precise degree of microbial mixing: molecular assays identify mixed-species consortia, but imaging often shows segregated aggregates with limited direct intermingling, underscoring the need for spatially resolved diagnostics.[1,46,47]

These dense, mixed-species communities not only coordinate metabolism and virulence but also create ideal conditions for horizontal gene transfer (HGT) through conjugation, transformation, and transduction.[44,57,65] In addition, biofilms also favor enhanced rates of spontaneous resistance mutations in part driven by oxidative stress and amplified under subinhibitory antibiotic concentrations.[46,66–68] Experimental evolution studies demonstrate that biofilm populations fragment into smaller subgroups,[69] enabling fixation of beneficial mutations and clonal interference between resistant lineages.[57] This accelerates diversification and drives the emergence of MDROs, consistent with estimates that 30% of biofilm-positive wounds contain AMR strains, many of which exhibit multidrug resistance.[70] Thus, biofilms both tolerate antibiotics and actively evolve under therapeutic pressure.

Alongside antimicrobial tolerance, biofilms also manipulate and exploit host immunity to further entrench infection. Neutrophils and macrophages are readily recruited but cannot penetrate the EPS, leading them to release proteases and ROS extracellularly, which damages surrounding tissue without eradicating infection.[21,45,46] At the same time, QS-regulated toxins lyse immune cells,[1,45] releasing DNA and actin that fortify the matrix. Macrophages exposed to *S. aureus* biofilms often shift toward dysfunctional M2-like phenotypes with impaired bactericidal capacity, prolonging ineffective inflammation.[45] Together, these host responses damage ECM components and angiogenic factors, stalling tissue repair and cementing wound chronicity.[71]

Despite recognition of biofilms as adaptive fortresses that promote chronic wound persistence and AMR,[26] much of our understanding still derives from *in vitro* and *in vivo* animal studies that only partially reflect human disease.[1,44,47,48,56,58] Detecting biofilms in clinical settings also remains challenging as standard culture methods often underestimate microbial diversity, miss slow-growing strains, and fail to capture spatial heterogeneity.[1,44,45,47,48] While advanced tools such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight, next-generation sequencing, and *in situ* fluorescence imaging have improved sensitivity, routine diagnostics remain variable, complex, and institution-dependent.[45,47,48] Therapeutic innovation is advancing (*e.g.,* microenvironmental modulation, EPS disruption, QS inhibition, phage- or peptide-based approaches, and combination strategies), but these approaches are not fully optimized or widely adopted in clinical practice.[44,46,55,58] Collectively, these trends represent a paradigm shift away from therapies targeting planktonic bacteria toward those tailored to the structural, regulatory, and metabolic complexity of chronic wound biofilms.

Mechanisms of AMR in wound pathogens

Biofilm tolerance and AMR are closely intertwined, as these structured microbial communities both shield resistant strains and facilitate the dissemination of resistance genes. The most concerning AMR pathogens in chronic wounds include methicillin-resistant *S. aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae*, vancomycin-resistant *Enterococcus*, multidrug-resistant *P. aeruginosa* (MRPA), and multidrug-resistant *Escherichia coli* (MREC).[72] The World Health Organization has classified antibiotic-resistant gram-negative bacteria as priority pathogens due to their ability to rapidly evolve resistance and transfer genes across species.[72] These organisms pose a formidable clinical challenge as conventional antibiotics and antiseptics often fail to penetrate bacterial cell walls at concentrations sufficient for eradication.[24,72]

AMR emerges when microbial pathogens persist despite antimicrobial exposure that would ordinarily inhibit or kill sensitive strains. In chronic wounds, this fosters persistent infection, delayed healing, and treatment failure. MRSA, detected in up to 34% of chronic wounds, remains a leading cause of both community- and healthcare-acquired infections.[72,73] Notably, resistance

often coexists with virulence. A longitudinal study of DFUs found that *S. aureus* isolates from non-healing ulcers carried resistance genes against aminoglycosides (*ant1*), tetracyclines (*tetA*), and macrolides (*ermA*), alongside virulence factors (staphylococcal enterotoxin C-2 [*sec2*] and enterotoxin A [*sea*]) that enhance immune evasion and tissue injury.[26] However, the mere presence of a resistance gene does not necessarily indicate that it is expressed or that it confers a phenotypic resistance profile. As such, integration of more complex gene expression analyses or antimicrobial susceptibility testing would provide clinically relevant data. This dual burden underscores the destructive and challenging nature of AMR strains in chronic wounds.

Three broad categories of resistance—intrinsic, acquired, and adaptive—contribute to AMR pathogen persistence (Fig. 4).[58,74] Intrinsic resistance arises from inherent bacterial traits, including efflux pumps, degradative enzymes (*e.g., β*-lactamases), and reduced membrane permeability.[30,58,74] For example, *P. aeruginosa* and *A. baumannii* employ efflux pumps (*e.g.,* MexAB-OprM and AdeABC) to expel antibiotics, while *P. aeruginosa* lipopolysaccharide (LPS) transport mutations reduce membrane permeability to tobramycin. Similarly, *K. pneumoniae* resists polymyxins through lipid A modification of its LPS layer.[75]

Acquired resistance develops when susceptible bacteria gain new resistance determinants, often through HGT or mutation.[58] The biofilm community reinforces acquired resistance by providing a stable niche where persister cells serve as reservoirs for resistance plasmids, surviving treatment and repopulating with resistant progeny. Plasmids, integrons, and transposons facilitate rapid gene exchange within polymicrobial wound communities

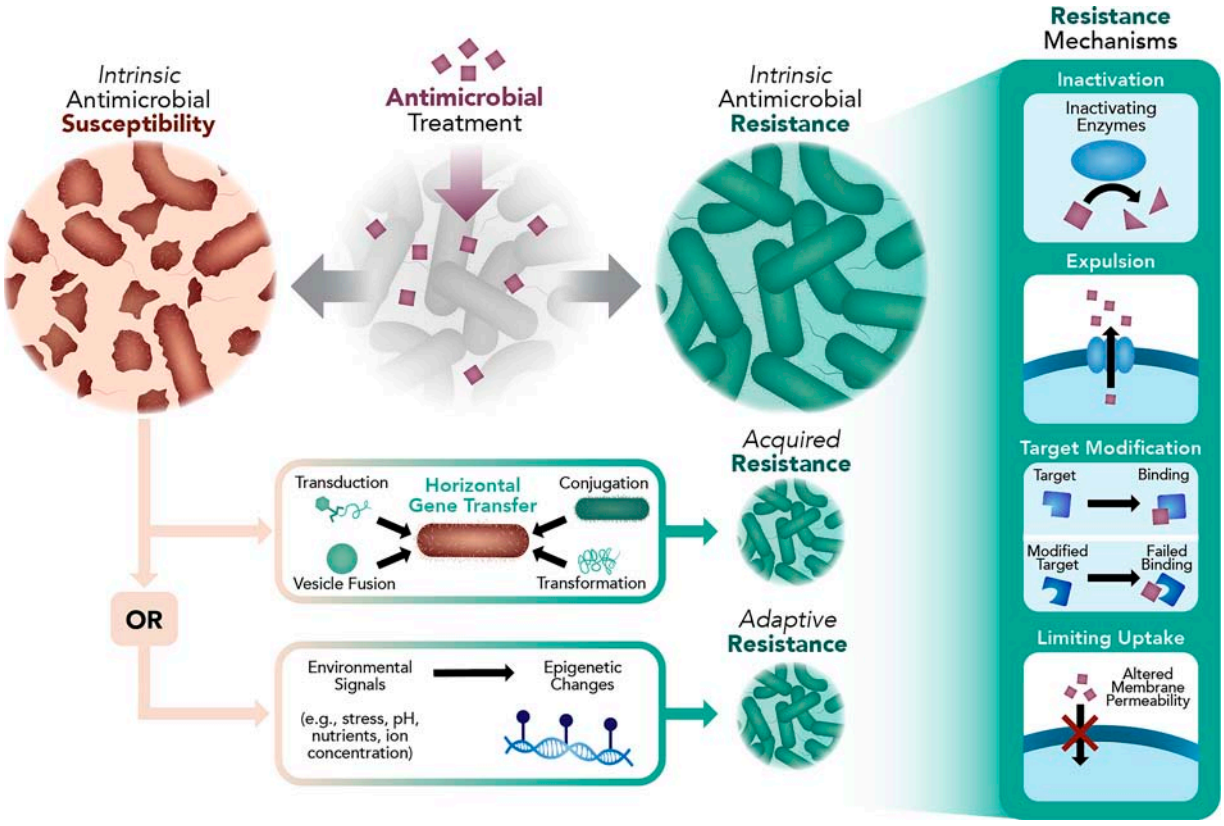

Figure 4. Mechanisms and development of antimicrobial resistance. In wound infections, antimicrobial resistance develops from an initially susceptible bacterial population (*intrinsic antimicrobial susceptibility*, left panel), whose susceptibility can be altered by antimicrobial exposure and environmental cues, including nutrient availability, pH, or ion concentration. Antimicrobial treatment imposes selective pressure, driving the transition from susceptibility to resistance through three routes: intrinsic, acquired, and adaptive resistance. *Intrinsic antimicrobial resistance* arises from pre-existing protective mechanisms inherent to certain bacterial species, which may be further enhanced by environmental or epigenetic factors. *Acquired resistance* occurs through horizontal gene transfer, including conjugation (direct cell-to-cell transfer), transduction (through bacteriophages), vesicle fusion, and transformation (uptake of extracellular DNA), enabling bacteria to acquire resistance genes. *Adaptive resistance* develops transiently in response to environmental stress and is often reversible once the stressor is removed. Resistance mechanisms include the production of inactivating enzymes (*e.g., β*-lactamases) that neutralize antimicrobial agents, efflux pump activation that expels drugs from the cell, modification of target sites to prevent effective antimicrobial binding, and reductions in membrane permeability. In biofilms, the EPS may also hinder antimicrobial penetration.

through multispecies cooperation, coaggregation, metabolic interactions, and QS.[76] MRSA exemplifies this process, having acquired the *mecA* gene that encodes penicillin-binding protein 2a and confers resistance to β-lactams.[74] Antimicrobial exposure further accelerates HGT, converting even benign commensals into opportunistic pathogens.[77]

Adaptive resistance reflects transient, reversible shifts in bacterial phenotype in response to environmental stressors such as altered pH, nutrient fluctuations, ion concentrations, or subinhibitory antimicrobial exposure.[74] In wound biofilms, the EPS matrix containing charged exopolysaccharides (*e.g., Pel*/*Psl* in *P. aeruginosa*), proteins (DNABII) and eDNA, physically impedes antibiotic diffusion and creates sublethal gradients.[78] Host-derived components in chronic wounds, including neutrophil extracellular traps, further thicken this barrier, preventing antibiotic access.[79] As such, pathogens, such as *P. aeruginosa* and *S. aureus*, adapt by downshifting metabolism, limiting drug penetration, and activating stress responses, thereby increasing survival under fluctuating wound conditions.[74,75]

Together, intrinsic, acquired, and adaptive resistance mechanisms, compounded by biofilm-associated tolerance, create formidable barriers to chronic wound healing and infection clearance.[5] The prevalence of these barriers is evident in whole-metagenomic sequencing studies of DFUs, which demonstrate that more than 50% contain resistance genes for aminoglycosides, macrolides, and β-lactams.[26] This finding underscores the urgent need for innovative AMR-limiting strategies in chronic wound care.

Clinical impact and burden of infected wounds

Wound infections impose a substantial burden on health care systems and patient outcomes, a challenge amplified by both AMR and biofilm formation (Fig. 5). Biofilms protect pathogens from host immunity and antimicrobials, enabling persistence, recurrence, and the spread of resistance genes. Resistant infections drive up health care costs through prolonged treatment durations, extended hospital stays, and reliance on alternative therapies that are often unavailable in resource-limited settings. In the United States, chronic wounds cost Medicare an estimated $28–32 billion annually, with surgical site infections (SSIs) and DFUs accounting for the highest expenditures.[18,80] In the United Kingdom, the mean direct cost of an unhealed wound is £13,700, with infection more than doubling this figure.[81] Although cost structures vary across health care systems, resistant wound infections consistently generate higher expenses in secondary and tertiary care settings, where prolonged care and specialized interventions are required.[73,77] The shift toward outpatient management has not lessened the burden, as the prevalence, recurrence, and chronicity of biofilm-driven drug-resistant wounds remain high.

Clinical outcomes mirror the economic strain. Patients with infected wounds typically experience more than double the length of hospital stay compared with uninfected patients (*e.g.,* 10.6 versus 5.6 days), and 30-day readmission rates are far higher (51.94 vs. 8.19 per 100 procedures).[82] These infections necessitate repeated antibiotic use, specialized dressings, surgeries, and intensive follow-up, straining hospital capacity and outpatient resources alike.

The consequences extend beyond wound care itself. AMR undermines the success of critical, immunosuppressive therapies such as chemotherapy, organ transplantation, and routine dental interventions that depend on effective infectious prophylaxis.[83] Postoperative and treatment-associated wounds in these vulnerable populations are particularly prone to MDRO infections, which heighten the risks of SSIs, sepsis, delayed recovery, and treatment failure.[2,16] Alarmingly, an estimated 70% of bacteria responsible for wound infections are resistant to at least one antibiotic, underscoring the convergence of AMR and biofilm tolerance as a pandemic-level threat.[2]

While health care systems bear substantial costs from chronic wound infection and AMR, the most profound toll is felt by patients (Fig. 5). Chronic, nonhealing infected wounds can severely limit mobility, particularly in lower extremity wounds, compromising independence and quality of life.[84] Infections raise the risk of sepsis, amputation (especially for diabetic wounds), and even death, with postoperative mortality rates nearly doubling in the presence of infection.[85] Skin and soft tissue infections are now the sixth leading cause of AMR-attributable deaths, posing significant challenges in both community and health care settings.[73] Beyond physical complications, ongoing pain, immobility, psychological distress, and social isolation are common, often compounded by financial hardship from lost income and out-of-pocket expenses.[84]

Given the substantial burden of AMR and biofilms in chronic wound care, urgent action is required. Comprehensive antimicrobial stewardship, strict infection control, and the development of novel wound management strategies are essential to mitigate resistance, disrupt biofilms, and reduce the far-reaching impacts of chronic wound infections.

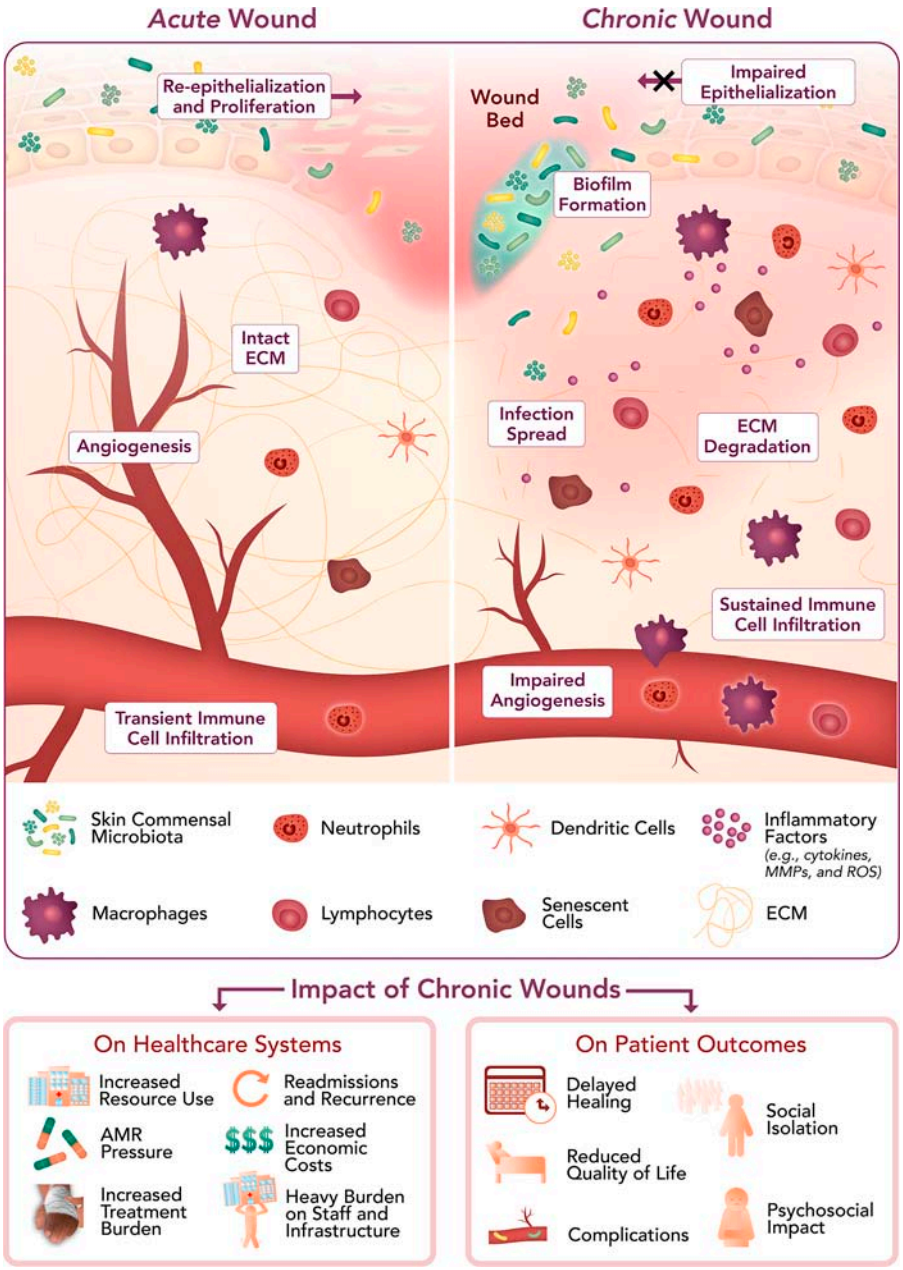

Figure 5. Impact of wound infection on health care systems and patient outcomes. Chronic wound infections are difficult to treat due to frequent biofilm formation, which impairs epithelialization and perpetuates inflammation through cytokine release and sustained activation of proinflammatory immune cells (*e.g.,* macrophages). These processes drive extracellular matrix degradation through matrix metalloproteinases (MMPs) and reactive oxygen species, while sustained immune cell infiltration and impaired angiogenesis limit new blood vessel formation, further delaying healing. Clinically, these infections result in prolonged hospitalizations, more frequent outpatient visits, and increased need for advanced wound therapies (*e.g.,* frequent dressing changes and intravenous antibiotics). This heightened treatment intensity increases both direct medical costs (*e.g.,* staff time, supplies, and inpatient care) and indirect costs (*e.g.,* lost productivity and disability), while excessive antibiotic use fuels the emergence of AMR. Recurrent infections and hospital readmissions add further strain on health care infrastructure and staffing resources. For patients, chronic wound infections are associated with delayed healing, high recurrence risk, and elevated mortality, particularly when complications such as cellulitis, sepsis, or amputation occur. Psychosocially, patients experience anxiety, depression, social isolation, and reduced quality of life, driven by immobility, pain, sleep disruption, and loss of independence. Together, these factors underscore the profound dual burden of wound infection on both health care systems and the individuals they serve.

Current and emerging diagnostic strategies

Accurate diagnosis and classification of wound infections are essential for effective management, optimization of healing, and reduction of AMR risk. Early and tailored interventions reduce the risk of chronicity, biofilm establishment, and resistance development. The use of clinical frameworks, such as Nonhealing, Exudate increase, Red friable tissue, Debris, Smell and Size increase, Temperature elevation, Os (bone) exposed, New breakdown, Erythema/Edema, Exudate, Smell, can help in distinguishing the presence of superficial localized

infection and deeper infection (Table 1). The assessment involves evaluating wound characteristics to help make informed decisions about appropriate wound treatment. For instance, a wound with excessive exudate, elevated temperature, and redness in the surrounding tissue is indicative of a deep tissue infection and is best treated with systemic antibiotcs.[86] Risk in patients with lower extremity wounds can be further stratified by the wound, ischemia, and foot infection system, which correlates infection severity with amputation risk and the likelihood of wound healing.[87,88]

Despite the application of these frameworks, additional diagnostics are needed to identify the culprit pathogens and help tailor treatment choices.[89] Current best practice[90] relies on a combination of serum inflammation biomarkers[91] and conventional tissue sample-based cultures, due to considerations of availability, cost, and the lack of robust comparative-effectiveness data.[98] However, these approaches are poor at detecting biofilms, which often go unrecognized until recalcitrance to antimicrobials signals their presence.[23] Biofilms not only hinder detection but also serve as reservoirs for resistance genes, through HGT,[65,95] compounding delays in diagnosis and limiting the effectiveness of standard approaches.

However, emerging novel advanced molecular diagnostic methods (*e.g.,* 16S rRNA sequencing and metagenomics) reveal greater microbial diversity and resistance gene profiles,[92] and adjunctive imaging modalities (*e.g.,* scanning electron microscopy,[93] fluorescence *in situ* hybridization,[93] computed tomography, magnetic resonance imaging,[94] and ultrasound[99]) help identify deep infections and complications.[96] Furthermore, rapid, point-of-care assays for biofilm-specific biomarkers offer promise for real-time identification of biofilm presence.[97,100] Once validated for routine clinical use, these advances will provide clinicians with more accurate microbial identification and aid them in making more tailored therapeutic decisions.

The current therapeutic landscape

Strong consensus exists that the most effective therapeutic approach to biofilm-infected wounds combines early antimicrobial intervention, appropriate antiseptic use, and thorough debridement alongside targeted antibiofilm treatments.[23] Antibiotic selection is typically empirical or culture-guided, yet efficacy is often short-lived because biofilm diminish drug penetration and effectiveness, particularly after debridement when they rapidly reform. Debridement, whether surgical (gold standard),[101] mechanical (gauze), enzymatic (papain, collagenase), ultrasonic, or biological (maggot therapy), remains essential for physically removing necrotic tissue and microbial biomass.[102] Still, identifying the most effective adjunctive antibiofilm targeted therapy remains a challenge as chronicity or recurrence of wounds is often not reversed. The wound treatment landscape is rich with antimicrobials (Table 2), but their performance against biofilms varies widely, as does their potential to potentiate AMR (Fig. 6). Moreover, in certain contexts, tissue disruption during debridement may inadvertently facilitate deeper penetration of bacteria, potentially promoting invasion into previously uninvolved wound compartments. This underscores the complex interplay between

Table 1. *Summary of current and emerging diagnostic strategies*

Category	*Approach*	*Clinical Utility*	*Limitations*
Current			
Clinical Assessment Frameworks[86–88]	NERDS, STONES	Bedside identification of superficial critical colonization and deep infection, respectively Guides early therapy decisions	Subjective; does not identify pathogens or resistance profile; cannot directly confirm biofilm presence
Standard Laboratory[89–91]	Serum inflammatory markers; conventional tissue/fluid cultures	Widely available; supports infection diagnosis; cultures enable antimicrobial susceptibility testing	Nonspecific biomarkers; reduced sensitivity in biofilm associated infections; slow turnaround; false negatives after antibiotics
Adjunctive Imaging Modalities[92–94]	SEM; FISH; CT MRI, ultrasound	Biofilm structure visualization; spatial localization of microbes; deep infection, abscesses, complications	Not routine; requires specialized equipment; limited availability; indirect biofilm detection; cannot identify species
Emerging			
Molecular Diagnostics[95]	16S rRNA sequencing; shotgun metagenomics	Culture-independent detection; identifies polymicrobial communities and resistance genes; reveals greater microbial diversity	Cost; limited standardization; turnaround time; may detect nonviable organisms
Rapid/Point of Care[96,97]	Biofilm-specific biomarker assays	Potential for real time detection; rapid	Requires further validation; not routine

CT, computed tomography; FISH, fluorescence *in situ* hybridization; MRI, magnetic resonance imaging; NERDS, Nonhealing, Exudate increase, Red friable tissue, Debris, Smell; rRNA, ribosomal ribonucleic acid; SEM, scanning electron microscope; STONES, Size increase, Temperature elevation, Os [bone] exposed, New breakdown, Erythema/Edema, Exudate, Smell.

Table 2. *Advantages and disadvantages of current antimicrobial treatments*

Treatment Modalities	*Product Advantages*	*Product Disadvantages*
Antibiotics[31] Examples: Quinolones, tetracyclines, aminoglycosides, cephalosporins	• Broad-spectrum useful against common wound pathogens • Topical antibiotics in the form of creams/ointments/dressings control localized wound infection	• Prolonged use fosters AMR • Often ineffective against biofilm • May require combination with debridement or advanced therapies
Metal-based antimicrobials[103,104] Examples: Silver, zinc, metal oxide nanoparticles	• Broad-spectrum antimicrobial activity against bacteria, fungi, and some viruses • Multiple mechanisms of antimicrobial action • Silver compounds have established safety profile • Some (particularly zinc) have additional wound healing benefits • Synergistic effects with other antimicrobials • Some formulations show antibiofilm efficacy	• Potential cytotoxicity at high concentrations • May delay wound healing • Environmental concerns regarding nanoparticle disposal • Variable clinical evidence base across different metal oxides • Regulatory challenges related to nanomaterial classification • Silver associated with AMR
Natural antimicrobials[105,106] Examples: Honey, chitosan, cellulose-derivatives and peptides	• Derived from natural sources including plants, animals, and microorganisms • Inhibit or kill microorganisms • Used in medicine, food preservation, and agriculture to control the growth of harmful microbes • Chitosan shows preclinical efficacy against biofilms	• Batch-to-batch variability is high leading to inconsistent potency • Instability and short half-life • Standardization challenges • Limited spectrum of activity • Poor efficacy • Less sustainable due to frequent dressing changes
Iodine-based antimicrobials[28] Examples: Povidone-iodine (PVP-I), liposomal iodine, cadexomer iodine	• Broad-spectrum antimicrobial activity • Low potential for development of AMR • Some formulations (particularly cadexomer iodine) also have debriding properties • Effective in biofilm reduction	• Potential for allergic reactions and skin irritation • Contraindicated in patients with thyroid disorders or those on lithium therapy • Require frequent dressing changes • Can be inactivated by wound exudate proteins
PHMB (polyhexamethylene biguanide)[107]	• Broad-spectrum antimicrobial activity • Low cytotoxicity at therapeutic concentrations • Sustained antimicrobial action • Effectiveness against biofilms • No known AMR to date	• Limited long-term safety data compared to silver and iodine • Potential for allergic reactions • Variability in PHMB concentration across different products • Not as effective in wound exudate
Nonmedicated wound dressings[108,109] Examples: Carboxymethylcellulose (CMC), dialkylcarbamoylchloride (DACC), hydroresponsive wound dressings	• No active/pharmaceutical component • Reduction of wound bioburden in a physical manner • Antibiofilm capabilities • Noncytotoxic • No risk of AMR	• Limited clinical data with no evidence of superiority over standard of care • May require combination with debridement or other advanced therapies
Nitric oxide therapeutics[110] Examples: Stimulators of endogenous NO, exogenous NO-releasing dressings, NO donor systems	• Broad-spectrum antimicrobial with antibiofilm properties • Regulate inflammation and wound healing • Low risk of AMR • Flexibility in delivery mode	• Short half-life, stability concerns • Cytotoxicity with some agents • Action highly dependent on contact time and concentration • Lacking in quality clinical efficacy evidence

AMR, antimicrobial resistance; CMC, carboxymethylcellulose; DACC, dialkylcarbamoylchloride; NO, nitric oxide; PHMB, polyhexamethylene biguanide; PVP-I, povidone-iodine.

Transparency and Disclosure: A portion of the content included in this table was generated using the generative AI tool available from: www.grok.com using the search prompt "create an overview of antimicrobial platform technologies used in advanced wound dressings that evolved during the last 20 years" and then reviewed and further modified by the authors for brevity, consistency, and flow.

microbial factors and host tissue responses in driving disease progression even in the setting of standard-of-care interventions.

Antibiotic-containing wound dressings, while clinically useful, are strongly associated with AMR development, especially when used long-term or inappropriately.[31] Similarly, silver-based agents are widely used but linked to emerging silver-resistant isolates,[32] and toxicity concerns with metal-based treatments can delay healing. Nanoparticles containing copper, zinc oxide, or gold show potent antimicrobial and antibiofilm effects by disrupting membranes, generating ROS, and interfering with metabolism.[17] However, concerns regarding toxicity, stability, and tissue accumulation remain.[31]

Antiseptics, such as povidone-iodine, polyhexamethylene biguanide, and honey provide broad-spectrum antimicrobial activity with a relatively low risk of resistance. However, their performance is reduced in highly exudative wounds, and standardized clinical guidelines are lacking.[17,31] Natural antibacterials, including plant extracts, essential

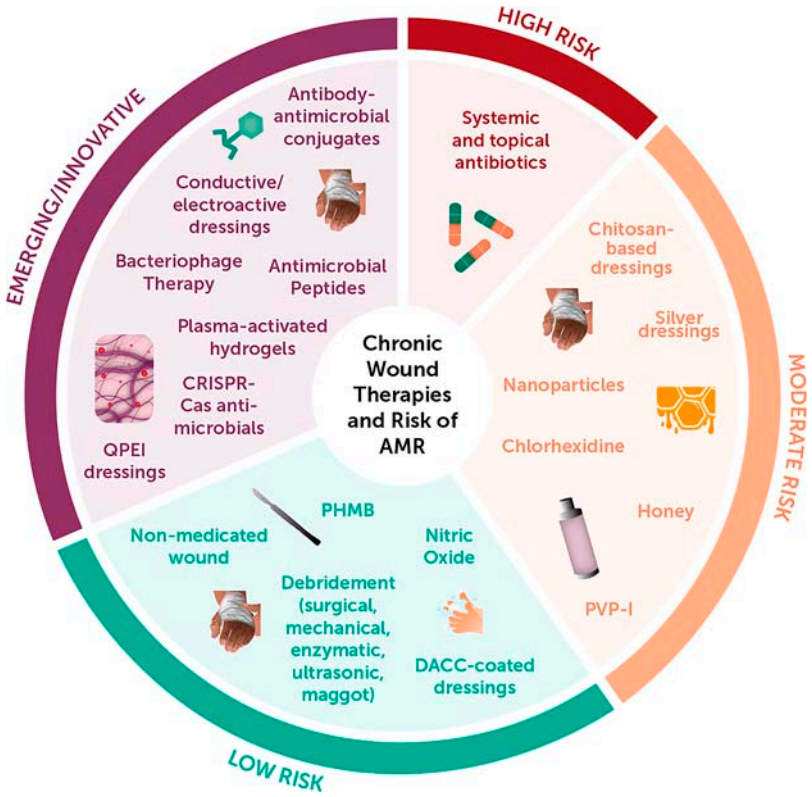

Figure 6. Comparison of AMR risk with current and emerging therapies. The pie chart classifies chronic wound therapies according to their risk of contributing to AMR, a critical concern in infection management. Therapies are divided into three categories: low risk (green), moderate risk (orange), and high risk (red). Low-risk therapies include nonmedicated wound care, debridement, polyhexamethylene biguanide (PHMB), nitric oxide, dialkylcarbamoyl chloride (DACC)-coated dressings, and povidone-iodine (PVP-I). These interventions carry minimal AMR potential due to their nonantibiotic mechanisms of action. Moderate-risk therapies, including chitosan-based or silver dressings, nanoparticles, chlorhexidine, and honey, may exert selective pressure on microbial populations, creating potential for resistance development. Moreover, systemic and topical antibiotics pose the greatest AMR risk due to their broad-spectrum activity and frequent use. Emerging innovative treatments (purple) include antibody-antimicrobial conjugates, antimicrobial peptides, conductive or electroactive dressings, bacteriophage therapy, plasma-activated hydrogels, CRISPR-Cas antimicrobials, and quaternary polyethyleneimine (QPEI) dressings. These technologies are highlighted for their low projected AMR risk profiles and potential to improve outcomes as they advance toward clinical application. This figure underscores the need to balance therapeutic efficacy with AMR risk when selecting chronic wound treatments.

oils, and fungal metabolites, are under investigation, though variable potency limits reliability.[31,111]

Nonmedicated wound dressings (NMWDs) mitigate infection risk and AMR pressure by physically removing bacteria from the wound environment.[108] Dialkylcarbamoyl-chloride–coated dressings, for example, bind and remove pathogens through hydrophobic interactions, lowering bacterial load at each dressing change. These dressings have been shown to significantly reduce levels of both *S. aureus* and *P. aeruginosa*, including AMR strains, making them a valuable option for biofilm control.[108] Other NMWDs, such as hydrofibers or superabsorbent polymer dressings, trap bacteria and prevent bacterial proliferation and reinfection.[108] Despite these benefits, NMWDs require frequent dressing changes since they do not actively kill bacteria.

Nitric oxide (NO)-based therapies offer antimicrobial and wound healing benefits by promoting vasodilation, angiogenesis, and immune modulation.[112] NO-releasing dressings enhance bacterial clearance and tissue regeneration, but efficacy is limited by short half-life and dependence on contact time and concentration.[110] Alternatively, chitosan-based dressings provide intrinsic antimicrobial activity and lower AMR risk via membrane disruption,[113] but their potency is lower than that of standard antimicrobials, which often necessitates concomitant nanoparticle use.[17,31]

Selecting optimal antimicrobial treatments is complex, as each of the available modalities carries unique benefits and limitations.[31] Stewardship is critical to reduce AMR risk,[114,115] particularly when using broad-spectrum antibiotics or silver formulations. While antiseptics, nanoparticles, and natural therapies show promise, limited clinical evidence, cytotoxicity concerns, inconsistent potency, and variable activity spectra constrain routine use.[32,114] These limitations underscore the urgent need for optimized formulations that more effectively

disrupt biofilms, preserve healing, and minimize AMR development.

Emerging therapeutic strategies

Beyond conventional antimicrobials, innovative strategies are emerging to combat wound infections, target biofilms, and minimize AMR risk. A selection of promising new technologies along with their benefits and drawbacks is discussed below (Table 3).

Antibody-antimicrobial conjugates represent an emerging technology that enables targeted delivery of antimicrobial payloads (*e.g.,* narrow-spectrum antibiotics) directly to infection sites. While earlier versions encountered resistance,[132] newer designs incorporate optimized antibodies, antibiotics, linkers, and payloads that maintain efficacy at low pH, improve cellular entry, and reduce AMR risk.[116] Preclinical studies demonstrate their potential to reduce biofilms in dermal wounds.[133]

Naturally derived AMPs,[134] though naturally limited by poor potency, toxicity, and instability, are being redesigned through artificial intelligence (AI). Generative AI models can identify peptide sequences with favorable properties, including sustained activity, low toxicity, and specificity against certain pathogens.[118] While computational simulations accelerate discovery and enhance accuracy, experimental validation remains essential to confirm efficacy.[119]

Environment-responsive nanofibers and hydrogels are also under development, engineered to release antimicrobial or antibiofilm agents (*e.g.,* silver and antibiotics) in a targeted fashion in response to wound pH and temperature.[120–122] Self-healing hydrogel dressings with stimuli-responsive release mechanisms, generated through reversible chemical or physical connections in a 3D network, enhance durability and precision, reducing AMR risk while improving activity against biofilm-embedded bacteria.[121] However, the effectiveness of this technique depends on the nanomaterial cargo design.

Bioelectric and electroactive dressings are also under development, leveraging the natural electrical gradients in wounds to enhance cell migration and healing.[123] When conductive polymers are integrated with antimicrobial and antibiofilm agents, multifunctional wound dressings emerge, though cytotoxicity and external power source requirements limit adoption. Self-powered piezoelectric and triboelectric-driven hydrogels are promising alternatives but underexplored.[135] A recent clinical study of a wireless electroceutical dressing showed efficacy toward reducing biofilms in acute trauma a burn wound infection; however, longer-term efficacy studies in chronic wounds are warranted.[136]

Polyethyleneimine (PEI)-based therapies represent another promising avenue, utilizing cationic polymers composed of branched or linear repeating ethyleneimine units. Functionalized quaternary ammonium groups along the PEI backbone create a positively charged, polycationic structure (QPEI), enabling strong binding to negatively charged bacterial cell walls and membranes and subsequent cell lysis.[130] This physical mechanism of action ensures broad-spectrum antimicrobial activity and reduces the risk of resistance to QPEIs.[137] Importantly, QPEIs exhibit potent antibiofilm activity, preventing formation and destabilizing mature structured microbial communities, positioning them as promising candidates for infection control and wound management.[130,138,139] However, most evidence for QPEI antibiofilm efficacy comes from *in vitro* or short-term *in vivo* studies, and the incorporation of QPEI into wound dressing materials must be carefully controlled to preserve material integrity.[139]

Plasma-activated antimicrobial systems utilize cold atmospheric pressure ionized gas (plasma) to generate reactive oxygen and nitrogen species (RONS) with potent antimicrobial and antibiofilm effects. Plasma-activated hydrogels are being developed to combat wound pathogens and enhance tissue healing. These hydrogels deliver longer-acting RONS (*e.g.,* peroxide and NO) to the wound while filtering out short-acting, destructive radicals, balancing bacterial killing and tissue protection.[124] However, the minimal biofilm eradication concentration for RONS is 5- to 40-fold higher than the minimal inhibitory concentration for planktonic pathogens, highlighting the need for combinatorial strategies to enhance biofilm disruption.[140]

Antimicrobial photodynamic therapy (aPDT) is based on delivery of photosensitizing agents to wounds, which upon light activation generate ROS that selectively targets dividing bacteria while sparing healthy tissue.[126] Despite its low resistance potential, early version of aPDT was hindered by short-lived antimicrobial effects and time-intensive administration.[127] Advances now include targeted photosensitizers,[141] self-illuminating[142] or wearable light delivery systems,[143] and combinatorial approaches leveraging advanced delivery systems and synergy with other antimicrobials to improve antibiofilm efficacy and durability.[144]

Over the past decade, clustered, regularly interspaced, short palindromic repeats (CRISPR)-CRISPR-associated protein systems have emerged

Table 3. *Advantages and disadvantages of emerging technologies*

Treatment Modalities	*Product Advantages*	*Product Disadvantages*
Synthetic antimicrobial biologics[116,117] Examples: Engineered proteins, synthetic antimicrobial peptides, chimeric proteins, antibody-antibiotic conjugates	• Enhanced stability • Potential antimicrobial plus wound healing promotion • Reduced risk of AMR • Possibility for highly specific targeting of problematic pathogens • Potential for controlled sustained release	• More complex storage and stability requirements • Potential for immunogenic responses with repeated use • Limited activity against intracellular pathogens • Antibiofilm potential dependent on carrier for targeted delivery
AI-designed antimicrobial peptides and proteins[118,119] Examples: Protein structure prediction-based antimicrobials, target-specific antimicrobial peptides, multifunctional AI-designed peptides	• Rational design minimizing unwanted toxicity • Potential for unprecedented antimicrobial efficacy • Ability to address specific resistance mechanisms • Shorter development timeline than traditional discovery methods • Potential for peptides with multiple beneficial functions	• Limited real-world validation compared to naturally derived peptides • Manufacturing challenges for complex peptide structures • Potential for unexpected immunogenicity
Responsive antimicrobial nanofiber networks and hydrogels[120,121] Examples: Infection responsive, thermo-responsive	• On-demand antimicrobial release to reduce unnecessary exposure • High surface-to-volume ratio enhancing effectiveness • Customizable mechanical properties matching wound tissue • Enhanced patient comfort through responsive properties • Potential for integration with wound monitoring technologies • Electroconductive dressing show antibiofilm activity	• Potential for premature or delayed triggering in complex wounds • Manufacturing complexity ensuring consistency • Storage stability challenges • May require specialized training for optimal clinical use
Bioelectric and electroactive dressings[121–123] Examples: Electrically conductive, self-adjusting,	• Release antimicrobial or antibiofilm agents in a targeted fashion • Self-healing • Stimuli-responsive release mechanisms • Reversible chemical or physical connections in 3D networks • Enhanced durability and precision • Reduction in AMR risk • Antibiofilm potential	• Effectiveness depends on nanomaterial cargo design • Early-stage development
Plasma-activated antimicrobial systems[124,125] Examples: Plasma-activated hydrogels, plasma-particle composites, plasma-polymer hybrid materials, on-demand plasma generating devices	• Multimodal antimicrobial mechanisms • Potential effectiveness against antibiotic-resistant organisms • No chemical residues or AMR • Some wound healing • Adjustable intensity for different wound types • Antibiofilm potential	• Requires specialized equipment • Limited penetration depth in complex wounds • Potential tissue damage with improper parameters • Limited long-term clinical data
Antimicrobial photodynamic therapy[126,127] Examples: Targeted photosensitizers, self-illuminating systems, photosensitizer-antimicrobial conjugates, wearable light delivery systems	• Nonantibiotic approach reducing resistance concerns • Potential for repeated use without diminishing efficacy • Ability to target antibiotic-resistant organisms • Dual action: antimicrobial plus potential biofilm disruption • On-demand activation for controlled treatment	• Limited tissue penetration depth for some wavelengths • Requires precise light dosimetry for optimal effect • Potential photosensitivity issues for some formulations • Requires patient/provider education for proper use
CRISPR-based antimicrobial systems[128,129] Examples: CRISPR-edited cell-based therapies, endogenous CRISPR antimicrobial systems, conjugative plasmid delivery systems	• Targets specific pathogens • Avoids harming beneficial bacteria • Can reverse AMR • Scalable with potential for mass customization	• Requires precise design and testing • Potential immunogenicity in humans • Potential off-target effects in nontarget cells • Major technical hurdles for delivery *in vivo* • Bacteria may develop anti-CRISPR mechanisms • Strain-dependent variability in antibiofilm potential
PEI-based antimicrobials[130,131] Examples: Coated wound dressings, nanoparticle antimicrobial delivery, nonviral vector for nucleic acid delivery, PEI-containing composite materials	• Broad-spectrum antimicrobial action • Membrane disruptor mechanism effective against biofilm • Low risk of AMR • Can be modified to customize function • Synergistic killing effects when combined with other antimicrobials	• Must be modified to avoid cytotoxicity • Environmental impact due to poor biodegradability • Aggregation can hinder consistency of delivery • Limited to external applications

AI, artificial intelligence; AMR, antimicrobial resistance; CRISPR, clustered, regularly interspaced, short palindromic repeats; PEI, polyethyleneimine.

Transparency and Disclosure: A portion of the content included in this table was generated using the generative AI tool available from: www.grok.com using the search prompt "create an overview of the most promising future antimicrobial platform technologies used in advanced wound dressings" and then reviewed and further modified by the authors for brevity, consistency, and flow.

as powerful tools for precise genome editing, capable of eliminating resistant and virulent pathogen strains by targeting specific DNA sequences and inducing lethal double-stranded DNA breaks.[128,129] However, the clinical translation of CRISPR-based antimicrobials is largely constrained by efficient and targeted delivery. Phage-delivered CRISPR constructs targeting drug-resistant pathogens have shown promise in selectively removing virulent and drug-resistant strains from mixed bacterial populations.[145,146] Alternative delivery platforms, including conjugative plasmids, can achieve efficient transfer in planktonic bacteria but face significant limitations in biofilm settings due to uneven distribution. In addition, nanoparticle-based carriers may introduce immunogenicity and toxicity challenges.[128]

KEY FINDINGS

- Chronic wounds are a growing global health and economic burden, affecting 1–2% of the population and disproportionately impacting elderly and diabetic patients.
- Antimicrobial resistance (AMR) exacerbates the challenges of chronic wounds, further compounded by biofilms that act as physical barriers for pathogens, shielding them from antimicrobial treatments and the host immune response.
- Gaining a greater understanding of the biofilm microbiome and its role in driving wound chronicity and AMR will inform new innovative treatment modalities.
- Current antimicrobials face significant limitations, including cytotoxicity, potential for AMR development, and inconsistent potency limiting clinical acceptance.
- Advanced wound treatment modalities currently under development hold promise in providing multifunctional capabilities for antimicrobial/antibiofilm action and enhanced wound healing while minimizing the risk of AMR.

Together, these emerging technologies represent a paradigm shift from conventional antimicrobial therapies toward precision-engineered, multimodal strategies that can physically, chemically, or genetically target chronic wound infections while minimizing AMR. Despite promising preclinical results, clinical translation remains limited by a multitude of challenges. Many of these therapeutic approaches are validated using highly controlled *in vitro*,[44,133] *ex vivo*,[147] and *in vivo*[148] systems that only partially replicate the complexity of the biofilm-infected chronic wound environment. While animal models provide the necessary host factors, experimental time scales lack sufficient length to accurately reflect the chronicity of human wounds.[1] Moreover, no single approach can address all aspects of the dysregulated systems at play, making demonstration of efficacy challenging.[149] As such, longer-term clinical studies are needed to determine the best approaches to maximize patient benefit.

Moving beyond the preclinical stage and into the clinic can have additional challenges in scalability, stability, regulatory approval, and cost-effectiveness. The therapies most likely to reach clinical practice will require evidence of superiority over existing treatments both in terms of healing times and reduced risk of AMR, be easy to administer in an outpatient setting, and demonstrate cost savings over the course of treatment. Despite the breadth of emerging technologies, the future of advanced therapies to treat chronic wound infection is still uncertain. Therefore, continued innovation will be essential to bridge the gap between technological potential and patient-centered clinical impact.

INNOVATION

Increasing adoption of antimicrobial stewardship in wound care has led to more timely diagnosis of infection and identification of wound pathogens, supporting tailored treatment decisions and reducing unnecessary antibiotic use that fuels AMR. At the same time, improved diagnostics hold promise for real-time detection of biofilm presence, composition, and antimicrobial susceptibility. Emerging wound care strategies aim to disrupt biofilm integrity, restore host immune function, and accelerate wound healing, while simultaneously limiting the development of AMR. Highly targeted delivery of antimicrobials may further enhance their effectiveness against biofilm-protected bacteria and resistant strains, while minimizing negative impacts on wound healing.

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editing (equal); R.F.: Conceptualization (equal), writing—original draft (supporting), and writing—review and editing (equal).

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Abbreviations and Acronyms

AI = artificial intelligence
AMP = antimicrobial peptides
AMR = antimicrobial resistance
aPDT = antimicrobial photodynamic therapy
ASPs = antibiotic stewardship programs
Cas = CRISPR-associated protein
CRISPR = clustered, regularly interspaced, short palindromic repeats
DACC = dialkylcarbamoylchloride
DFUs = diabetic foot ulcers
DNA = deoxyribonucleic acid
ECM = extracellular matrix
eDNA = environmental deoxyribonucleic acid
EPSs = extracellular polymeric substances
HGT = horizontal gene transfer
LPS = lipopolysaccharide
MDRO = multidrug-resistant organism
MMP = matrix metalloprotease
MREC = multidrug-resistant *Escherichia coli*
MRPA = multidrug-resistant *Pseudomonas aeruginosa*
MRSA = methicillin-resistant *Staphylococcus aureus*
NERDS = Nonhealing, Exudate increase, Red Friable Tissue, Debris, Smell
NMWDs = nonmedicated wound dressings
NO = nitric oxide
PEI = polyethyleneimine
PHMB = polyhexamethylene biguanide
PVP-I = Povidone-Iodine
QPEI = quaternary ammonium polyethyleneimine
QS = quorum sensing
RONS = reactive oxygen and nitrogen species
ROS = reactive oxygen species
SSI = surgical site infection
STONES = Size increase, Temperature Elevation, Os (Bone) Exposed, New breakdown, Erythema/Edema, Exudate, Smell