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Polymicrobial Biofilms in Diabetic Foot Ulcer: Pathogenic Synergy, Clinical Challenges, and Therapeutic Perspectives

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Diabetic foot ulcer (DFU) is a chronic complication of diabetes mellitus characterized by delayed healing and high recurrence rates. A key factor contributing to therapeutic failure is the presence of polymicrobial biofilms. Interspecies interactions within these biofilms promote EPS matrix thickening, antimicrobial resistance, immune evasion, and prolonged inflammation, thereby increasing the risk of amputation. This narrative review evaluates the clinical and therapeutic implications of these interactions and highlights strategies for effective management. A comprehensive search was conducted using Scopus, PubMed, and Google Scholar for studies published between 2015 and 2025. From 283 articles identified, 37 met the inclusion criteria and were reviewed. Results confirmed that interspecies interactions significantly impair wound healing and therapeutic outcomes in DFU. These interactions strengthen microbial persistence and delay tissue repair, underscoring the limited efficacy of conventional antimicrobial therapies. Clinically, debridement and negative pressure wound therapy (NPWT) remain the most widely supported interventions, while antibiotic–antibiofilm combinations and enzymatic EPS-disrupting agents show promising synergistic effects. Emerging modalities, including phage therapy, quorum sensing inhibitors, and smart dressings, are under development but largely restricted to preclinical studies. The clinical and therapeutic impact of interspecies interactions highlights the need for multimodal DFU management. Integrating mechanical and antibiofilm approaches with future innovations such as microbiota-guided personalized therapy and adaptive wound dressings may improve healing outcomes and reduce the risk of severe complications.

Keywords: Antibiofilm therapy; Diabetic foot ulcer; Interspecies interaction; Polymicrobial biofilm; Quorum sensing; Wound microbiota.

Diabetic foot ulcer (DFU) is an open wound occurring on the feet of individuals with diabetes mellitus, characterized by poor healing capacity and high susceptibility to infection. It represents one of the most serious chronic complications of diabetes. Diabetic foot ulcers (DFUs) affect approximately one-third of the half a billion individuals living with diabetes worldwide. More than half of DFU cases progress to infection[1] and 14–24% of these require lower-limb amputation due to osteomyelitis and other severe complications.[2]

Infections in DFU are rarely caused by a single microbial species. Instead, the majority of cases involve polymicrobial communities composed of various aerobic and anaerobic species that coexist and interact within biofilm structures. Common microbial combinations in

DFU infections include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, as well as anaerobes such as *Bacteroides fragilis* and *Peptostreptococcus* spp.. [2] These interspecies interactions not only enhance resistance to antimicrobial treatments but also influence virulence patterns, gene expression, and metabolic activity of the constituent microbes.[3]

Clinically, microbial infections—particularly those involving complex communities organized within biofilm structures—play a critical role in exacerbating the wound condition. Microbial biofilms provide physical and biochemical protection, enabling pathogens to evade host immune responses and resist antimicrobial treatments. This contributes significantly to therapeutic failure and recurrent infections.[4]

Biofilm resistance to therapy can also result from physiological adaptations of the constituent bacteria. These changes are often triggered by the complex microenvironment of chronic wounds, which exerts selective pressures that drive microbial persistence. In addition, exposure to topical or systemic treatments can sometimes promote the dominance of more virulent or competitive species. For instance, in a chronic wound model, *Pseudomonas aeruginosa* was observed to outcompete other members of the polymicrobial community under treatment pressure.[3]

Recent studies have demonstrated that the microbial bioburden—both in terms of quantity and community composition—correlates directly with changes in inflammatory biomarkers, oxidative stress levels, and wound healing capacity in DFU. These changes collectively contribute to delayed wound healing.[5] Such findings suggest that biofilm communities in DFU are not merely passive bystanders but actively shape the immunological landscape of the wound in ways that are unfavorable for recovery.

This narrative review aims to highlight the importance of advancing knowledge on the characteristics of polymicrobial biofilms and interspecies interactions in DFU infections, as a foundation for developing more effective antibiofilm therapeutic strategies. Specifically, the objectives of this review are to: (1) summarize current understanding of polymicrobial biofilm characteristics and the dominant pathogenic species involved in DFU infections; (2) provide an overview of interspecies interactions within polymicrobial biofilms in DFUs; and (3) discuss the clinical and therapeutic implications of these interactions.

MATERIALS AND METHODS

Search Strings and Databases

The articles for this literature review were collected through systematic searches of online databases, including Scopus, PubMed, and Google Scholar, conducted on May 15, 2025. The initial search strategy employed the keywords *"polymicrobial biofilm"* and *"diabetic foot ulcer"* in each database. Only English-language articles published between 2015 and 2025 were included, focusing on microbial interspecies interactions within polymicrobial biofilms in DFU, as well as their clinical and therapeutic implications. Data management and article screening were facilitated using Rayyan.ai software. The screening process was performed independently and in a blinded manner by all authors, and any disagreements were resolved through discussion.

Eligibility Criteria

The screening process involved an initial evaluation of article titles and abstracts, followed by full-text assessment of the eligible studies to confirm their relevance for inclusion in this review.

The inclusion criteria were as follows:

1. Studies discussing the pathogenic profiles and characteristics of polymicrobial biofilms in DFU
2. Studies addressing interspecies interactions among pathogens within these biofilms
3. Studies exploring the clinical and therapeutic implications of polymicrobial biofilms in DFU
4. Studies related to chronic wounds were also included, provided they discussed pathogens commonly associated with DFU

The exclusion criteria were:

1. Studies deemed irrelevant to the research objectives
2. Studies with incomplete or inaccessible full texts
3. Studies not written in English
4. Studies discussing interspecies interactions within biofilms or their clinical and therapeutic implications but not involving pathogens associated with DFU

Although this study is a narrative review, the screening process was structured according to the principles adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to enhance the transparency and quality of article selection.

RESULTS

A total of 283 articles were initially identified through databases, including 263 from Google Scholar, 17 from Scopus, and 3 from PubMed. During the title and abstract screening phase, 108 articles were excluded due to irrelevance. The remaining articles underwent full-text screening, resulting in the exclusion of 138 articles that did not meet the predefined inclusion criteria. Ultimately, 37 articles were included in this literature review (Figure 1, 37 articles listed in Appendix).

The selected literature consistently reported that DFU harbor complex polymicrobial biofilms composed of aerobic and anaerobic bacteria as well as fungi. Commonly identified species

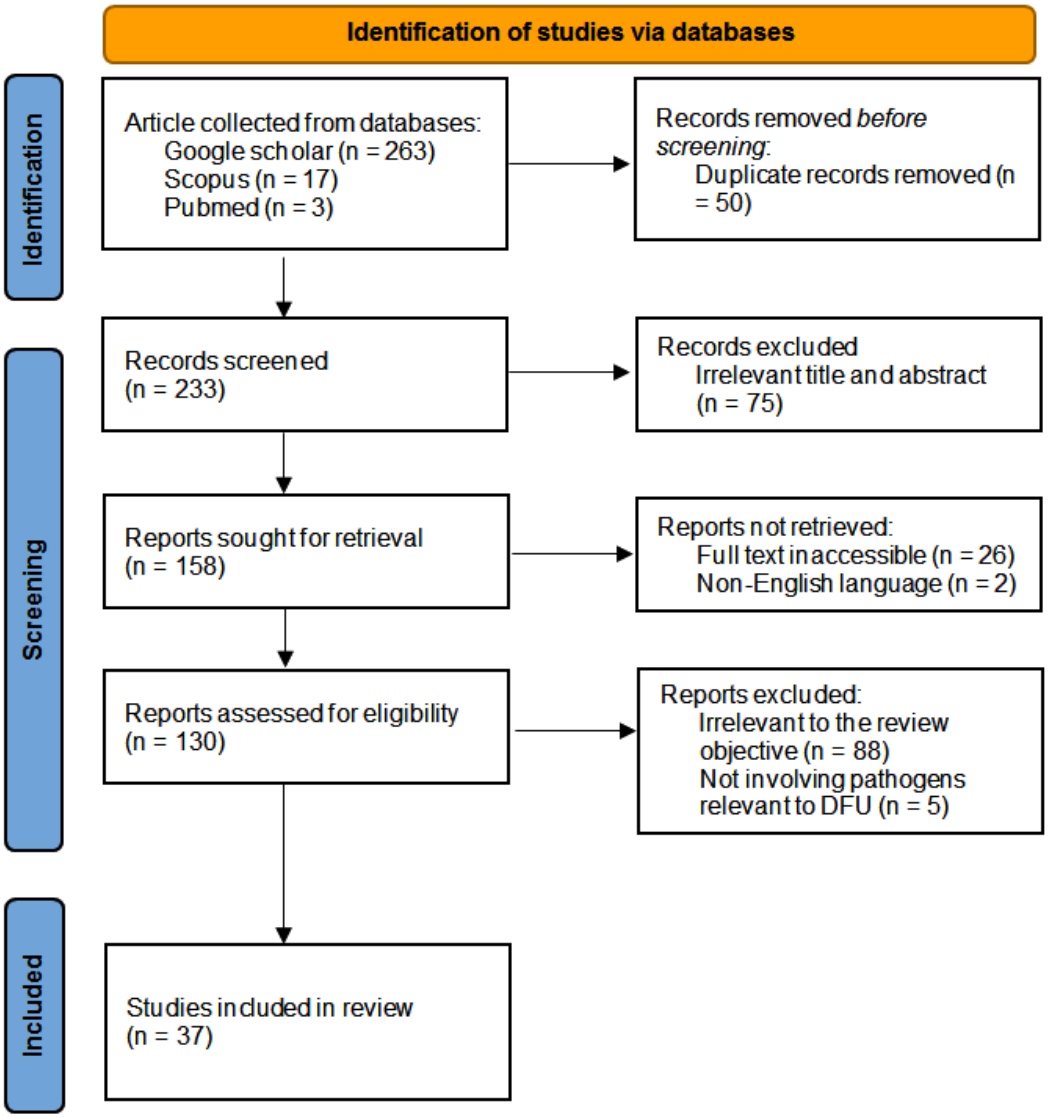

Fig. 1. Flowchart of literature screening and selection process

included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Finegoldia magna*, *Peptoniphilus asaccharolyticus*, and *Candida albicans*. The reviewed studies highlighted that these polymicrobial biofilms are characterized by a dense extracellular polymeric substance (EPS) matrix, interspecies quorum sensing—particularly via autoinducer-2—and frequent occurrence of horizontal gene transfer. Such features were recurrently associated with enhanced antimicrobial resistance, persistent inflammation, and delayed wound healing in DFU.

DISCUSSION

Characteristics of Polymicrobial Biofilm in DFU

Polymicrobial biofilms in DFU represent highly heterogeneous microbial communities. These communities may include aerobic, anaerobic, and facultative anaerobic bacteria, as well as fungi, all of which interact within a complex three-dimensional structure embedded in an extracellular polymeric substance (EPS) matrix.[6]

Polymicrobial biofilms possess evolutionary advantages over single-species biofilms due to their ability to engage in interspecies communication mediated by quorum sensing (QS). This communication is mediated by the signaling molecule autoinducer-2 (AI-2), which can be produced and recognized by both Gram-positive and Gram-negative bacteria, enabling broad-spectrum coordination of community-wide behaviors. QS enables microbes to coordinate the production of EPS, secretion of proteolytic enzymes, and the timing of biofilm dispersal.[7] QS also allows microbes to adapt more effectively to fluctuating conditions within chronic wounds, such as changes in pH, oxygen levels, or nutrient availability. Furthermore, QS facilitates the collective regulation of enzymes that degrade antibiotics or neutralize antagonistic compounds, thereby reinforcing communal resistance.[8]

Through the QS mechanism, different species of microbes in community may produce thicker and more structurally complex EPS, because each species may contribute complementary components to the EPS matrix. The EPS matrix

Table 1. Microbial Species in DFU Biofilm and Their Interspecies Interaction

Species	Role in Biofilm	Type of Interaction
Staphylococcus aureus	Forms SCV, survives in biofilm, engages in antagonistic/cooperative interactions[23]	Dynamic interplay with *P. aeruginosa*[24,25]
Pseudomonas aeruginosa	Strong EPS producer, secretes toxic molecules (HQNO, pyocyanin), dominates interactions[22]	Antagonistic toward *S. aureus* (via HQNO)[22]; cooperative with *E. faecalis*[28]
Enterococcus faecalis	Strengthens EPS matrix, metabolic cross-feeding[28]	Multifaceted interactions (synergistic and antagonistic) with other microbial species[29]
Escherichia coli	Contributes to mixed biofilm formation, acts as a metabolic supporter[19]	Commensalism or metabolic cooperation with anaerobes[18]
Acinetobacter baumannii	Aggravates inflammation and resistance, associated with suppressed host response[14]	Exploits wound environment, synergizes inflammatory response with others[14]
Finegoldia magna	Anaerobe, enhances biofilm stability, produces proteolytic enzymes[15]	Cooperative with Peptoniphilus and *E. faecalis* in anaerobic communities[15]
Peptoniphilus asaccharolyticus	Synergistic with other anaerobes, reinforces biofilm structure and inflammation[15]	Cooperative, synergizes in complex anaerobic biofilms[15]
Candida albicans	Forms inter-kingdom biofilm, cooperates with bacteria (*S. aureus*)[30,31]	Cooperative, builds joint matrix with bacteria; enhances collective virulence[6]

in polymicrobial biofilms serves as both a cellular adhesive and a primary protective barrier that shields microbial communities from antibiotics and host immune responses. This EPS matrix also enables horizontal gene transfer (HGT) between bacteria, thereby accelerating the evolution of multidrug resistance. Mechanisms such as conjugation and transformation occur more frequently within the dense, structured environment of a biofilm compared to planktonic conditions.[9]

Polymicrobial biofilms in chronic wounds such as DFU exhibit a complex three-dimensional architecture characterized by the abundant production of extracellular polymeric substances (EPS). This thick and structurally intricate matrix plays a crucial role in shielding bacteria from antibiotics and host immune responses. Findings from the nBioChip study demonstrated that polymicrobial biofilms composed of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* form dense microcolonies embedded within a rich EPS matrix. The biofilm thickness reached up to 150 µm, and the microcolony volumes varied widely from 500 to 10,000 µm³, highlighting a high degree of structural heterogeneity.[10]

In addition, the DFU substrate—rich in fibrinogen, collagen, and necrotic tissue—contributes to the incorporation of wound proteins into the EPS matrix, resulting in a hybrid structure known as a "biofilm-protein" complex. Several studies have further suggested that such biofilms may facilitate the formation of microbial amyloids—beta-sheet-rich fibrous proteins that reinforce the biofilm structure and significantly enhance resistance to antimicrobial agents.[11] This

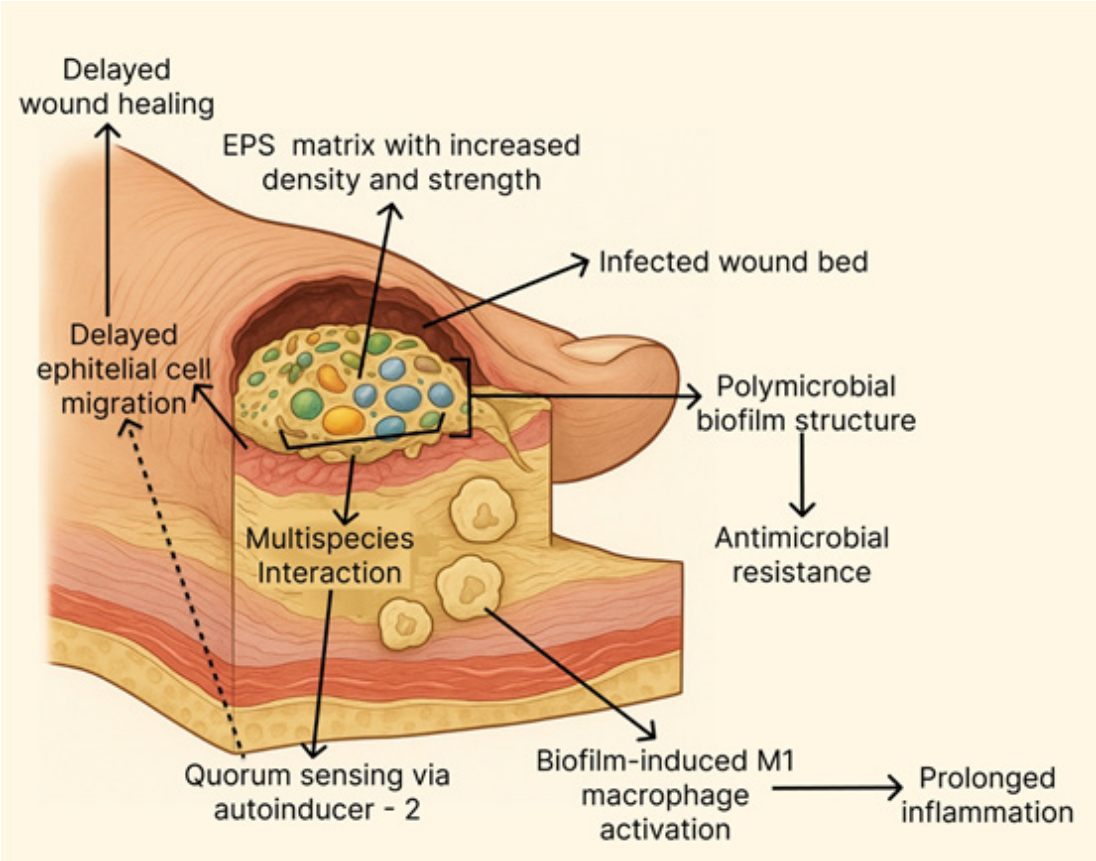

Fig. 2. Schematic illustration of a polymicrobial biofilm in a DFU. The image illustrates how multispecies microbial interactions contribute to the formation of a dense extracellular polymeric substance (EPS) matrix, enhance antimicrobial resistance, prolong inflammation, and delay epithelial cell migration (created using Figma).

Table 2. Therapeutic Strategies against DFU Biofilm

Therapeutic Strategy	Mechanism of Action	Level of Evidence	Advantages	Limitations
Mechanical Debridement[39]	Removes necrotic tissue and disrupts biofilm physically	Clinical	Effective in debriding wounds and promoting healing	Requires frequent application, May not be suitable for all wound types
Enzymatic EPS Disruption (e.g., DNase, Dispersin B)[41]	Degrades EPS matrix to expose embedded bacteria	In vitro	Targets biofilm structure and reduces glucose levels, promotes angiogenesis and collagen deposition	Primarily tested in vitro, requires further clinical validation
Antibiofilm and Antibiotics Combination[10,42]	Targets bacteria within biofilm with synergistic action	In vitro, clinical	Enhanced efficacy against MDR bacteria, biofilm disruption	Limited large-scale clinical trials, potential for resistance development
Quorum Sensing Inhibitors[43]	Interrupts microbial communication to weaken biofilm	In vitro	Targeted action against biofilm formation, reduced virulence	Limited in vivo evidence, potential for off-target effects
Negative Pressure Wound Therapy (NPWT)[40]	Reduces exudate, promotes granulation and perfusion	In vivo, clinical	Faster healing, reduced wound size, increased granulation tissue	Potential complications like pain and bleeding, cost
Phage Therapy[44,45]	Lyses specific bacteria within biofilm using bacteriophages	In vitro, early clinical	Specific targeting of pathogens, effective against MDR	Limited large-scale clinical trials, regulatory challenges
Smart Dressings with Bioactive Agents[46]	Delivers agents directly to wound, adapts to environment	In vitro, early clinical	Multifunctional, promotes healing, reduces infection	Limited long-term data, potential for allergic reactions
Personalized Therapy based on Microbiota Profile[47]	Adjusts therapy based on the primary pathogens and the condition of the wound	early clinical	Customized approach, potentially higher efficacy	Requires advanced diagnostics, higher cost

integrated matrix is particularly difficult to remove through conventional wound cleaning methods.[8]

The phenomenon of priority effects within polymicrobial communities in DFU infection can also significantly influence the strength, structure, and pathogenic potential of the resulting biofilm. In this context, the order of microbial colonization plays a critical role in determining the final biofilm architecture, resistance traits, and virulence. For instance, early colonization by *Candida* has been shown to modify the wound surface in a way that facilitates the subsequent adhesion of pathogenic bacteria such as *Staphylococcus aureus*.[12] These priority effects may partly explain the interpatient variability observed in the characteristics of DFU biofilms, even when the same microbial species are present.

Dominant and Pathogenic Species in DFU Biofilms

Identifying the dominant pathogenic species in DFU biofilms is essential to understand their roles in persistence, virulence, and treatment resistance. This knowledge forms the basis for examining interspecies interactions and their clinical implications. The following section outlines the dominant microbial species within each major group frequently associated with DFU biofilms.

Aerobic bacteria

Acinetobacter baumannii is an aerobic bacterium frequently detected in DFU infections, often at relatively high abundance. This pathogen has emerged as a major clinical concern due to its remarkable ability to persist in harsh environments, form robust biofilms, and display resistance to multiple classes of antibiotics. Metagenomic analyses have identified A. baumannii in approximately 23% of patients with type II diabetes and have associated its presence with impaired systemic inflammatory responses.[13] Moreover, the coexistence of *A. baumannii* and *Staphylococcus aureus* within biofilm communities has been shown to produce denser and more treatment-resistant biofilm structures compared to monomicrobial biofilms.[14]

Anaerobic bacteria

Various anaerobic genera such as *Peptoniphilus* and *Finegoldia* have been identified in significant abundance through molecular techniques. These organisms were previously underrecognized due to the limitations of conventional culture methods. However, metagenomic studies have demonstrated that anaerobes are frequently associated with deep, non-healing ulcers and may play a crucial role in the pathogenesis of persistent biofilms.[15,16] These bacteria are often located in the deepest layers of the biofilm, where they are shielded from oxygen penetration, making them particularly difficult to eradicate using conventional therapies. Moreover, anaerobes may interact synergistically with aerobic bacteria to create a stable microenvironment that is highly resistant to external interventions.[15,17]

Facultative anaerobic bacteria

Staphylococcus aureus is one of the most dominant species involved in DFU infections. It is detected across various stages of infection, acting either as a primary pathogen or as part of a polymicrobial community. This pathogen is well known for producing a wide array of toxins and enzymes that facilitate biofilm formation and enhance resistance to treatment. Metagenomic studies have frequently identified *S. aureus* alongside coagulase-negative *Staphylococcus* species within biofilm communities of chronic wounds.[16]

Pseudomonas aeruginosa is another highly relevant Gram-negative bacterial species in the context of DFU. It is well known for its ability to form complex, antibiotic-tolerant biofilms and for its competitive dominance over other microbial species within chronic wound communities. *P. aeruginosa* produces various exotoxins and pigments that interfere with wound healing processes while conferring a colonization advantage. Notably, in chronic wound models, *P. aeruginosa* has been shown to outcompete other members of the polymicrobial community under treatment pressure.[18]

Escherichia coli and other members of the Enterobacteriaceae family—such as *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter* spp.—are also frequently isolated from DFU, either as single-species infections or as part of polymicrobial communities. *E. coli* has been consistently identified in both culture-based and metagenomic studies and is often associated with deeper wounds and prolonged ulcer duration.[16,19] *Klebsiella pneumoniae* and *Proteus mirabilis* are commonly found in polymicrobial biofilms of

patients with chronic ulcers and are characterized by high levels of antibiotic resistance, particularly to carbapenems.[20]

Several other Gram-positive bacteria, such as *Enterococcus faecalis* have also been identified within DFU biofilms and are known to participate in synergistic biofilm formation. Although *Enterococcus* species alone tend to produce relatively weak biofilms, their co-existence with other bacteria—such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*—has been associated with significantly increased biofilm thickness and biomass.[14]

Fungi

Recent research has demonstrated that fungal species play an important role in the biofilm ecosystems of DFU, alongside both aerobic and anaerobic bacteria. A longitudinal study involving 100 patients with neuropathic DFU reported that 79% of wounds were positive for fungal presence, with some harboring up to 20 different fungal species. Among these, *Candida albicans* was the most commonly identified fungus and showed a significant association with wound necrosis and an increased risk of amputation.[21]

Interspecies Microbial Interactions in DFU Biofilms

In DFU biofilms, microbial interspecies interactions play a pivotal role in determining the structure, resilience, and pathogenicity of the wound environment. These interactions are not merely passive cohabitations but involve complex processes of communication, competition, and cooperation among diverse bacterial and fungal species (Table I). Several notable types of interspecies interactions identified from the collected articles are outlined below.

Dynamic Interplay Between *Staphylococcus aureus* and *Pseudomonas aeruginosa*

The interaction between *Staphylococcus aureus* and *Pseudomonas aeruginosa* within chronic wound biofilms, including DFU, is one of the most complex and extensively studied interspecies relationships.

This interaction is generally antagonistic in nature. *P. aeruginosa* produces inhibitory molecules such as HQNO (4-hydroxy-2-heptylquinoline-N-oxide), which disrupts the respiratory chain of *S. aureus* cells. As a result, *S. aureus* is forced to shift into a small colony variant (SCV) phenotype—characterized by slow growth and reduced metabolic activity. Although this shift appears to weaken the bacterium, the SCV form is actually more resistant to antibiotics, many of which are most effective against metabolically active cells.[22]

Interestingly, although HQNO is generally considered lethal to *S. aureus*, the presence of albumin—a protein abundant in wound exudate and serum—can neutralize a portion of the toxic quorum sensing molecules released by *P. aeruginosa*. Albumin binds to HQNO and other toxic compounds, thereby mitigating their lethal effects on *S. aureus*. As a result, under certain conditions, these two pathogens can coexist within chronic wound biofilms.[22]

Recent studies have also shown that excessive alginate production by *P. aeruginosa* can enhance *S. aureus* survival by contributing to the EPS matrix, which offers physical protection from immune clearance and antibiotic exposure.[23,24] A study by DeLeon et al. (2014) demonstrated that when *Staphylococcus aureus* is co-cultured with *Pseudomonas aeruginosa* in a wound model, the precise spatial arrangement significantly influences antibiotic resistance. *S. aureus* cells embedded deeper within the biofilm or positioned near the exopolysaccharide-rich matrix produced by *P. aeruginosa* exhibited greater tolerance to gentamicin compared to those grown in monoculture.[25]

The coexistence between *Pseudomonas aeruginosa* and *Staphylococcus aureus* is also mediated by interspecies quorum sensing (QS) signals. *S. aureus* protein A has been shown to directly interact with the surface of *P. aeruginosa* cells, facilitating bacterial attachment and contributing to the stabilization of the biofilm community.[26]

Multifaceted Interactions of *Enterococcus faecalis* with Other Microbial Species

Enterococcus faecalis exhibits multifaceted interactions with other microbial species within chronic wound biofilm communities, demonstrating both synergistic and antagonistic behaviors.

One of the most notable synergistic mechanisms is metabolic cross-feeding, wherein *E. faecalis* utilizes hemoproteins derived from *S. aureus* via its GelE enzyme to enhance aerobic

respiration and promote co-biofilm formation, highlighting the metabolic interdependence among species.[27]

In addition, *E. faecalis* can enhance the survival of co-infecting pathogens by modulating the host immune response. This occurs through suppression of the NF-êB signaling pathway in macrophages, which reduces proinflammatory cytokine production and thereby facilitates colonization by pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa*, leading to more persistent infections.[27]

Other findings indicate that *E. faecalis* can also act cooperatively by contributing to EPS formation in mixed-species biofilms. In co-culture experiments with *P. aeruginosa*, *E. faecalis* was shown to induce the expression of exopolysaccharide synthesis genes (*pslA, pelA,* and *algD*) in *P. aeruginosa*, resulting in thicker and more treatment-resistant biofilms.[28]

Conversely, not all interactions are cooperative. Some studies report that *E. faecalis* exerts antagonistic effects against *S. aureus* by secreting bacteriocins or by altering the microenvironment—for example, through changes in pH or redox potential.[29]

These contrasting observations suggest that the role of *E. faecalis* within polymicrobial communities is context-dependent, potentially influenced by the composition of coexisting species, environmental conditions within the wound, and host immune factors.

Cooperative Interactions of *Escherichia coli* with *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Escherichia coli has been identified as a prevalent organism in DFU biofilms and is often found coexisting with other pathogenic species. According to Park et al. (2019), *E. coli* frequently co-colonizes with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, forming a polymicrobial community that contributes to deeper tissue invasion and chronicity of the wound. These interspecies associations are thought to support the persistence of *E. coli* by facilitating access to nutrients and enhancing biofilm stability.[19]Bottom of Form

Interkingdom Interactions Between *Candida albicans* and *Staphylococcus aureus*

Interkingdom interactions also play a crucial role in the biofilm communities of DFU. *Candida albicans*, for example, has been shown to interact with *S. aureus* through the fungal adhesin Als3p, which facilitates co-colonization and enhances resistance to antimicrobial agents.[30] Als3p enables *S. aureus* to adhere to *C. albicans* hyphae, allowing the bacterium to persist longer within wound tissues. Notably, *C. albicans* quorum sensing activity can modulate the expression of the *agr* quorum sensing system in *S. aureus*, thereby indirectly enhancing its virulence.[31]

The concept of "priority effects" also influences the interaction between *Candida albicans* and *Staphylococcus aureus*. When *C. albicans* colonizes first, it alters the nutritional niche and surface availability, promoting subsequent *S. aureus* adhesion and virulence. The glucan matrix produced by *C. albicans* further protects *S. aureus*, particularly against antibiotics such as vancomycin.[6] Conversely, if *S. aureus* establishes itself first, the resulting biofilm community exhibits distinct gene expression profiles and enhanced antifungal tolerance.[12]

Commensal–Pathogen Interactions in DFU Biofilm Communities

In silico metabolic modeling has demonstrated that biofilm communities in chronic wounds exhibit mutualistic behavior, characterized by substrate sharing and complementary metabolic functions. For example, *Peptoniphilus asaccharolyticus*, although often classified as a commensal bacterium, can supply amino acids and secondary metabolites that support the growth of other species, promoting the development of more mature and resilient biofilms.[32] This suggests that even numerically minor species may play essential roles in maintaining community stability and function.

Clinical Implications of Polymicrobial Biofilms in DFU

Polymicrobial biofilms in DFU present complex clinical challenges because of the protective functions of the extracellular polymeric substances (EPS) matrix. Within these communities, the EPS not only restricts antibiotic penetration and shields microorganisms from host immune defenses but also supports the survival of diverse species under hostile conditions and facilitates interspecies transfer of resistance genes.[33] This collective protection contributes to markedly

increased antibiotic resistance in DFU biofilms, arising not only from genetic determinants but also from the reduced metabolic activity of biofilm-embedded bacteria and limited antibiotic diffusion through the EPS. Consequently, antibiotic monotherapy is frequently ineffective.

Biofilm dispersion adds another layer of complexity in polymicrobial DFUs. Under antibiotic pressure or environmental changes, microbial cells from multiple species may detach from the biofilm and spread within the wound or into systemic circulation, heightening the risk of persistent infection, sepsis, and aggravated local inflammation.[34] Paradoxically, inappropriate therapeutic interventions—such as exposure to subinhibitory antibiotic concentrations—can stimulate dispersion across species and worsen the clinical outcome.

Polymicrobial biofilms in DFU also pose significant clinical challenges due to the combined effects of species diversity, spatial architecture, and interspecies interactions. In vivo studies have shown that mixed communities of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* exhibit markedly higher resistance to antimicrobial therapies than single-species biofilms, even under high-dose systemic antibiotic exposure.[35] This tolerance is reinforced by phenotypic adaptations such as *S. aureus* small colony variants, which display increased antibiotic resistance and enhanced survival under inflammatory and hypoxic conditions typical of DFU.[36,37]

In addition, multidrug-resistant strains of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *P. aeruginosa*—often resistant to carbapenems and aminoglycosides—further undermine empirical treatment, with resistance frequently mediated by horizontal gene transfer in the extracellular DNA-rich biofilm environment.[13]

These dynamics disrupt the balance of the wound microbiota, where the dominance of opportunistic pathogens like *S. aureus* and *P. aeruginosa* impairs tissue regeneration and prolongs inflammation.[21] The involvement of anaerobes and fungi such as *Candida* exacerbates the condition through interkingdom interactions that promote the development of dense, treatment-resistant biofilms.[30] Collectively, these factors highlight how polymicrobial interactions drive wound chronicity, complicate management, and increase the risk of adverse outcomes in DFU.

Mechanistic Basis of Clinical Implications: Immune Dysregulation in DFU Biofilms

Building upon the clinical challenges described above, it is important to understand the underlying mechanisms by which polymicrobial biofilms disrupt host immunity. Immune dysregulation represents a key mechanistic driver that sustains chronic inflammation in DFU and further impedes effective healing.

Polymicrobial biofilms in DFU exert profound clinical effects through multiple interconnected mechanisms (Figure 2). Biofilms trigger the activation of pro-inflammatory macrophages (M1), leading to excessive production of cytokines such as TNF-á and IL-6, as well as elevated levels of matrix metalloproteinases (MMPs). These mediators degrade the extracellular matrix, hinder epithelial cell migration, and prolong the inflammatory phase, thereby sustaining the chronic nature of the wound and impairing effective healing.[38]

Interspecies quorum sensing mediated by autoinducer-2 (AI-2) plays a central role by promoting cooperative microbial behaviors that enhance EPS matrix production, strengthen biofilm structure, and increase antimicrobial resistance.[7] The resulting EPS matrix provides physical protection for the multispecies community within the infected wound bed and further contributes to delayed epithelial migration and impaired inflammatory resolution.[9]

Collectively, these mechanisms explain how polymicrobial biofilms directly lead to delayed wound healing, persistent inflammation, and therapeutic failure in DFU.

Overview of Therapeutic Strategies for Polymicrobial Biofilm Management in DFU

The therapeutic strategies summarized in Table II represent findings from the articles included in this narrative review. These approaches highlight both conventional and emerging interventions that have been investigated for managing biofilms in DFU. However, it should be noted that other potential therapeutic strategies may exist but were not captured in this review, as they might be documented under different keywords or

terminologies. This represents a limitation of the present study, reflecting the scope of the literature search strategy.

Management of polymicrobial biofilms in DFU requires strategies that address the unique challenges of interspecies interactions, including increased resistance to antimicrobials, enhanced extracellular polymeric substance (EPS) production, and suppression of host immune responses.

Among the available strategies, mechanical debridement and negative pressure wound therapy (NPWT) remain clinically robust. Mechanical debridement directly reduces biofilm biomass and disrupts the dense EPS matrix, thus improving antimicrobial penetration and immune recognition.[39] NPWT has additional benefits in reducing exudate and promoting granulation tissue formation, which is critical in wounds where polymicrobial biofilms delay healing.[40] Together, these modalities provide an essential foundation for wound bed preparation in complex DFU.

To specifically counteract microbial synergy and heightened resistance within polymicrobial biofilms, antibiotic–antibiofilm combinations are a promising adjunct. This approach enhances efficacy against multidrug-resistant species and facilitates biofilm disruption.[9] Importantly, their use in combination with debridement or NPWT could improve outcomes by both reducing microbial load and weakening EPS-protected communities.

Similarly, enzymatic EPS-disruption therapies (e.g., DNase, dispersin B) target the protective biofilm matrix, exposing embedded bacteria to antibiotics and immune clearance.[41] These combinations are particularly relevant where thick EPS layers and cooperative resistance mechanisms hinder treatment efficacy.

Innovative therapies such as phage therapy, quorum sensing inhibitors, and bioactive smart dressings also hold potential in tackling polymicrobial biofilms. Phage therapy offers targeted lysis of bacterial species, which could selectively reduce dominant pathogens within multispecies biofilms while sparing commensals.[44,45] Quorum sensing inhibitors interfere with interspecies communication, thereby weakening coordinated resistance and virulence traits.[43] Smart dressings that deliver bioactive agents directly to the wound microenvironment further enhance local antimicrobial activity and modulate inflammation.[46] While these remain limited by scarce large-scale clinical evidence, they represent promising adjuncts to conventional methods in polymicrobial contexts.

Looking forward, personalized therapies based on microbiota profiling may provide customized interventions tailored to the specific polymicrobial composition of the wound.[47] Such strategies could optimize therapy against dominant resistant species while restoring protective microbiota, although cost and diagnostic demands remain barriers.

In conclusion, clinicians should prioritize clinically validated strategies—mechanical debridement and NPWT—as the cornerstone of management, complemented by antibiotic–antibiofilm combinations and EPS-disrupting agents, while gradually integrating advanced therapies to overcome the complex resilience of polymicrobial biofilms in DFU.

Future Perspectives in Polymicrobial Biofilm Disruption Strategies for DFU

Current strategies such as mechanical debridement and NPWT remain central in clinical management, but their limitations—including frequent applications, high cost, and tissue trauma—highlight the need for more accessible, multifunctional approaches. Future research should explore the integration of bioactive smart dressings with natural antibiofilm compounds (e.g., flavonoids, terpenoids, alkaloids), which could offer cost-effective solutions to reduce biofilm burden, particularly in resource-limited settings.[39,40,46]

To address the resilience of polymicrobial biofilms, priority should be given to therapies that disrupt the EPS matrix and suppress cooperative microbial resistance. Antibiotic–antibiofilm combinations and enzymatic EPS-degrading agents (e.g., DNase, dispersin B) enhance antibiotic penetration and biofilm disruption, while quorum sensing inhibitors targeting cross-species signals such as Autoinducer-2 (AI-2) may weaken collective defenses.[9,41,43] These approaches are particularly relevant in polymicrobial settings, where EPS thickening and interspecies communication enhance resistance.

Looking ahead, personalized therapy based on microbiota profiling represents a promising direction to tailor interventions according to patient-specific wound microbial communities.[47] Advanced 3D biofilm models and wound organoids will be essential to evaluate these strategies under conditions that closely mimic DFU environments.[48] In parallel, innovations such as adaptive "smart" dressings capable of responding to local wound pH or oxygen tension could provide dynamic, patient-centered solutions to overcome the persistence of polymicrobial biofilms.[9]

Limitations

This review has several limitations. First, potential search bias may have occurred since the literature search was restricted to specific keywords, which could have led to omission of relevant studies published under different terminologies. Second, the narrative design inherently lacks the methodological rigor of systematic reviews or meta-analyses, and thus does not provide a quantitative synthesis of evidence. Third, only articles published in English were included, which may have excluded important findings reported in other languages. Finally, the included studies were heterogeneous in terms of research design, detection methods, and outcome measures, limiting direct comparisons and generalizability of the findings.

CONCLUSION

Polymicrobial biofilms in DFU pose a significant clinical challenge due to interspecies interactions that reinforce biofilm resilience. Such interactions promote the formation of a thicker and more robust EPS matrix, impair host immune responses, prolong inflammation, and ultimately delay wound healing. These features explain the limited efficacy of conventional antimicrobial therapies and underscore the urgent need for integrated approaches that specifically address microbes in their biofilm state.

Based on current evidence, mechanical debridement and NPWT remain essential clinical tools, particularly when combined with antibiotic–antibiofilm therapies or EPS-disrupting agents to enhance biofilm clearance. Adjunctive strategies, including smart dressings with bioactive compounds and quorum sensing inhibitors, offer additional potential but require further validation. Clinicians should therefore adopt a multimodal approach that balances proven efficacy with the practical constraints of cost, accessibility, and patient safety.

Future research should advance the development of natural antibiofilm agents, enzymatic EPS disruptors, and quorum sensing inhibitors, while leveraging more physiologically relevant models such as 3D biofilms and wound organoids for translational testing. Personalized strategies guided by rapid metagenomic profiling of wound microbiota represent a promising frontier for customizing therapy to patient-specific microbial communities. Together, these innovations are critical to improving healing outcomes, reducing infection recurrence, and lowering the risk of limb amputation in patients with DFU.

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Conflict of interest

The authors declare that they have no financial or personal conflicts of interest that could have affected the outcomes of the research presented in this manuscript.

Data availability

This statement does not apply to this article.

Ethics statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed consent statement

This study did not involve human participants, and therefore, informed consent was not required

Clinical trial registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not applicable.

Author contributions

T. Hertiani (Principal Author): Conceptualization, supervision, writing - review and editing, projecting administration, funding acquisition; K.K. Ummah (First Author): Conceptualization, writing - original draft, methodology, investigation, formal analysis, data curation, resources, visualization, validation, software; T. Nuryastuti (Second Author): Writing - review and editing, supervision.

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