**Abstract**

**Background:**  
Fungal biofilms are complex, structured microbial communities that contribute significantly to persistent infections, particularly in immunocompromised individuals and in the context of medical device colonization. Their intrinsic resistance to conventional antifungal agents and poor detectability in clinical diagnostics underscore the need for innovative approaches in both identification and treatment.

**Objective:**  
To critically explore the current limitations in the diagnosis of fungal biofilms and highlight emerging therapeutic strategies targeting their unique structural and metabolic features.

Biofilm-associated infections pose diagnostic challenges due to the scarcity of specific biomarkers and the inability of standard laboratory protocols—largely optimized for planktonic cells—to detect sessile fungal communities. Advances in imaging, omics technologies, and metabolic profiling offer potential, yet remain limited to research settings due to cost, complexity, and lack of standardization. On a mechanistic level, biofilms exhibit enhanced antifungal tolerance through multifactorial processes, including dense extracellular matrices, stress-induced phenotypes, metabolic heterogeneity, and hypoxic microenvironments. These conditions reduce drug penetration and efficacy, leading to therapeutic failure even in drug-susceptible strains.

Recent studies have identified critical molecular determinants of biofilm fitness, such as ERG251-dependent ergosterol biosynthesis, and the role of extracellular vesicle cargo in coordinating matrix assembly and antifungal evasion. Novel antifungal agents, including glucan synthase inhibitors and triterpenoids, show improved activity against biofilm-embedded cells in preclinical models. Adjunctive strategies—such as the use of biofilm-disrupting enzymes, hypoxia-targeted molecules, or combinatory regimens—represent promising avenues currently under investigation.

**Conclusion:**  
Fungal biofilms demand a paradigm shift in diagnostic and therapeutic approaches. Integrating biofilm-aware strategies into clinical practice is essential to improve patient outcomes. Continued research into biofilm-specific targets and drug delivery systems is imperative to address this growing public health threat.

**Keywords:** fungal biofilms, antifungal resistance, diagnostics, therapeutic innovation.