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Antifungal therapy of *Candida* biofilms: Past, present and future

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ABSTRACT

Virtually all *Candida* species linked to clinical candidiasis are capable of forming highly resistant biofilms on different types of surfaces, which poses an additional significant threat and further complicates therapy of these infections. There is a scarcity of antifungal agents, and their effectiveness, particularly against biofilms, is limited. Here we provide a historical perspective on antifungal agents and therapy of *Candida* biofilms. As we reflect upon the past, consider the present, and look towards the future of antifungal therapy of *Candida* biofilms, we believe that there are reasons to remain optimistic, and that the major challenges of *Candida* biofilm therapy can be conquered within a reasonable timeframe.

1. Introduction

Different *Candida* species are considered to be opportunistic pathogenic fungi, capable of causing infections in humans ranging from superficial to invasive candidiasis [1]. Candidiasis is among the most common fungal infections, and its incidence has increased in the last few decades mostly as a result of a growing population of at-risk individuals, including both medically- and immune-compromised patients [1,2]. Although *Candida albicans* is still responsible for the majority of *Candida*-related infections, non-*albicans Candida* species including *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and most recently *C. auris* have become increasingly important as causative agents of candidiasis [3–5]. Invasive candidiasis typically carries high levels of mortality, as its diagnosis and treatment are still problematic [6,7].

A majority of manifestations of infections caused by *Candida* spp are associated with a biofilm etiology [5,8,9]. The ability of different species within this genus to adhere to inert and biological surfaces and subsequently form biofilms has been well established during the last couple of decades and, among pathogenic fungi, *Candida* spp. are the most frequently associated with biofilm formation [5,8,9]. *Candida* biofilms are a consortia of cells attached to a surface and enveloped within a matrix of self-produced exo-polymeric substances, reflecting optimal conditions for obtaining nutrients and disposing of metabolic waste products [10]. Furthermore, *Candida* cells within these highly organized structures are protected from a variety of environmental stresses, including host defenses and antifungal treatment, and as such biofilm development contributes greatly to the pathogenesis of candidiasis and greatly complicates treatment for these patients [5,9]. *Candida* biofilm formation also carries a significant financial burden to our health-care systems.

In *Candida* spp., the biofilm life-cycle occurs through multiple stages [11,12]. In an initial "colonization" stage, yeast cells attach to a surface. This is followed by a "proliferation" phase where cells replicate and grow, leading to an incipient organized structure. The subsequent "maturation" phase is characterized by the production of the extracellular matrix which encapsulates the entire structure. In *C. albicans*, but not other species such as *C. glabrata* and *C. auris*, the proliferation and maturation stages are also intimately linked with increased filamentation, leading to more robust biofilms with increased ultrastructural complexity [12,13]. Once the biofilm has reached maturity, a final "dispersion" stage involves the detachment of yeast cells from a fully matured biofilm so that the entire *Candida* biofilm developmental process can be fully replicated at a different site [14,15]. For example, this is often how invasive (or deep seated) candidiasis originates after dispersion and subsequent hematogenous dissemination from a biofilm formed inside a central venous catheter.

Seminal work from the Mitchell and Nobile groups, among others, has demonstrated that the *Candida* biofilm developmental process is also

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highly regulated at the molecular level [16–18]. This has been best studied and characterized in *C. albicans*, where over fifty transcriptional regulators have been described to control this process. Among these, a core set of nine key regulators give rise to a highly orchestrated and interconnected network in which the individual regulators control each other and approximately one thousand other target genes, including many other transcriptional regulators [17,19]. Interestingly, this *C. albicans* biofilm network is composed mostly of novel genes that evolved relatively recently during evolution, which may explain in part the success of this species as an opportunistic pathogen [17]. The entire biofilm developmental process is also finely controlled by quorum sensing mechanisms, with farnesol being a key molecule in this aspect [20].

As mentioned briefly above, from a clinical standpoint, one of the major adverse consequences of *Candida* biofilm formation is the fact that sessile cells show high levels of tolerance to antifungal therapy as compared to their planktonic counterparts [21,22]. To begin with, the antifungal arsenal is exceedingly short, mostly a consequence of the paucity of selective targets for antifungal drug development due to the close relatedness of human and fungal cells [21,23–25], and this lack of highly selective targets also contributes to the elevated toxicity frequently associated with antifungal therapy [23]. It has been demonstrated that the tolerance to antifungal treatment of *Candida* biofilms is multifactorial, with increased cell density, the biofilm matrix, the overexpression of efflux pumps, changes in ergosterol content, cell stress responses and the presence of persister cells being among the main factors contributing to the overall recalcitrance to antifungal drug treatment [26–28]. This exacerbated biofilm tolerance complicates the management of biofilm-associated *Candida* infections considerably and contributes to therapeutic failure. In addition, *Candida* biofilms afford fungal cells a safe haven, constitute reservoirs for persistent sources of candidiasis, and can also negatively affect the function of implanted devices [29]. As such, there is an urgent need for the development of novel antifungals and other alternative approaches for the treatment of *Candida* infections, and of biofilms in particular. Within this context, this review aims to illustrate the history of antifungal therapy against *Candida* biofilms and its limitations, while also highlighting potential new avenues that may offer renewed optimism for the development of novel effective strategies to combat their threat.

2. The past

Polyenes, azoles and echinocandins are the main classes of existing antifungal agents used for the treatment of *Candida* infections [23–25]. All of these classes were discovered and further developed during the past century, with echinocandins representing the newest class of approved antifungals about two decades ago [30,31].

The polyenes are the oldest class of antifungal agents, their discovery and development dating back to the 1950s, with amphotericin B being the first ever FDA-approved antifungal for the treatment of invasive fungal infections, including candidiasis [23,24]. These amphipathic compounds act as a sponge extracting ergosterol from the fungal cell membrane, leading to the formation of pores, causing leakage of cellular components and ultimately death of the fungal cell [32,33]. As such, members of this class are considered to be fungicidal agents and display broad spectrum of activity against medically-important fungi. However, their efficacy is severely compromised by their intrinsic toxicity, particularly nephrotoxicity, and also by infusion-related toxicity [23]. Despite its inherent drawbacks, amphotericin B remained the “gold standard” of antifungal therapy for decades, mostly due to the lack of viable alternatives. From the very early reports on biofilm activity, it was already demonstrated that cells within *Candida* biofilms show increased resistance against polyenes [22]. For example, amphotericin B was approximately 10 times less potent when tested under biofilm-versus planktonic-growing conditions, but these high concentrations needed to effectively kill biofilms are considered toxic and unsafe, effectively restricting its use [22]. Nevertheless, the Ghannoum group demonstrated that newer liposomal formulations of amphotericin B showed unique activity against *Candida* biofilms [34], which can be attributed to better penetration of these formulation across the biofilm matrix.

Azole derivatives was the next class of antifungals, developed mostly in the 1980s and 1990s [23,24]. They represent the largest class of antifungal agents used today in clinical medicine [35]. Azoles also target ergosterol, but by inhibiting its biosynthetic pathway, most specifically the cytochrome P-450 14-alpha lanosterol demethylase, and they are considered fungistatic drugs [23,25]. In particular, after its introduction in the 1980s, fluconazole rapidly became first line therapy against *Candida* infections, mostly due to its improved safety profile compared to amphotericin B. This time coincided too with the increase of oropharyngeal candidiasis seen in HIV-infected patients. However, the development of resistance and the decreased susceptibility of several *Candida* spp (*i.e. C. glabrata* and *C. krusei*) represented major challenges to the use of fluconazole for the treatment of candidiasis. Unfortunately, early work on *Candida* biofilms demonstrated their intrinsic resistance to azole derivatives, and in particular fluconazole, with minimum inhibitory concentration values as much as 1000 times higher than those obtained for planktonic populations [22], which severely restricted the clinical use of fluconazole (and other azole derivatives) for the treatment of biofilm-associated candidiasis.

Echinocandins, the newest class of antifungals, were first discovered in the 1970s, although their development took place mostly during the 1990s, and it was not until the early 2000s when caspofungin, the first FDA-approved member of this class, entered the market [25,30,31]. These drugs are a group of semisynthetic lipopeptide antibiotics which inhibit 1,3-β-D-glucan synthase, the key enzyme for the synthesis of glucan, the main structural component of the *Candida* cell wall [30]. Agents within this class display potent fungicidal activity against the majority of species within the *Candida* genus, which added to their excellent safety profile (the cell was is fungal specific and not present in mammalian cells), contributed to echinocandins becoming front-line therapy for the treatment of candidiasis [30]. However, the emergence of resistance, through mutations in the gene encoding the target enzyme, may limit their efficacy [36]. Interestingly, the development of echinocandins occurred at the very same time that different groups were starting their pioneering work on *Candida* (mostly *C. albicans*) biofilms. Early work in these academic laboratories in the late 1990s and early 2000s demonstrated the potent antifungal activity of physiological concentrations of echinocandins against *C. albicans* biofilms, with subsequent studies extended to other *Candida* spp [34,37,38].

3. The present

Unfortunately, during the last couple of decades research and development (R&D) on antifungal drugs has been mostly discontinued at a majority of large pharmaceutical companies, as big pharma has prioritized more profitable drugs to treat chronic conditions. As a result, antifungal R&D now largely relies on the efforts of much smaller biotechnology companies, with also much more limited financial resources [25]. Compared to anti-virals and anti-bacterials, there are relatively few companies aimed at developing the next generation of antifungal agents [25]. We note that, in the US, the GAIN (Generating Antibiotic Incentives Now), the Orphan Drug Act and the FDA’s Fast Track designation are all applicable to antifungal drug development. Also, the Qualified Infectious Disease Product (QIDP) designation is reserved for antibacterial and antifungal drug candidates intended to treat serious or life-threatening infections At the present time there are a handful of investigational agents at different stages of the antifungal development pipeline. Although the majority still target ergosterol (same as azoles) or 1,3-β-D-glucan (same as echinocandins), they also include some novel classes with novel targets and mechanisms [25,39,40]. Altogether, these new agents offer new hope for the treatment of

Candida infections; however, relatively little is known about their activity specifically against *Candida* biofilms.

Rezafungin (formerly CD101) is a new long-acting echinocandin with improved stability and extended half life as compared to the first generation drugs within this class, thereby potentially allowing for once a week dosing [41]. It is being developed by Cidara Therapeutics, which recently filed for and was granted Priority Review for rezafungin for the treatment of candidemia and invasive candidiasis. Consistent with previous studies of echinocandins, rezafungin is also active against *C. albicans* biofilms, as treatment with this drug both inhibits biofilm formation and is affective against mature biofilms [42]. Its activity against biofilms formed by other *Candida* species is presumed, although remains to be evaluated.

Also targeting 1,3-β-D-glucan synthase but structurally different to the echinocandins, Ibrexafungerp (formerly SCY-078, formerly MK-3118) is a new semi-synthetic terpenoid drug, currently being developed by Scynexis Inc [43]. Ibrexafungerp was recently approved for the treatment of vulvovaginal candidiasis in 2021, and as such represents the first approved drug within a novel antifungal class in more than 20 years. Regarding its anti-biofilm activity, somewhat unsurprisingly since it shares the same target with echinocandins, ibrexafungerp has been shown to display activity against biofilms formed by different *Candida* species, including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* [44], and most recently *C. auris* [45].

Belonging to an entirely new class of antifungals, with novel chemical structure, target and mechanism of action, fosmanogepix (formerly APX001) is a new orally available broad-spectrum antifungal agent [39,46] currently being developed by Amplyx Pharmaceuticals, which was recently purchased by Pfizer Inc. This new first-in-class small molecule antifungal is actually an N-phosphonooxymethyl prodrug which is rapidly and completely metabolized by systemic alkaline phosphatases to the active moiety, manogepix (APX001A, formerly designated E1210). Fosmanogepix inhibits the inositol acyltransferase Gwt1 which catalyzes and early step in the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway, thereby preventing GPI-anchored protein maturation, a process critical for linkages between different components of the fungal cell wall [39,46]. There is limited information on the activity of this new class of antifungals, but in an early study manogepix inhibited *C. albicans* adherence and biofilm formation in a concentration dependent manner among other virulence factors [47].

Other notable antifungals presently being developed include the tetrazoles VT-1129, VT-1161, and VT-1598 (Mycovia Pharmaceuticals, following its acquisition of Viamet Pharmaceuticals). Their molecular target is the same as for the azoles, 14-alpha-lanosterol demethylase [48], but these tetrazoles display increased selectivity for the fungal enzyme over mammalian cytochrome p-450 enzymes [48]. To our knowledge, their anti-biofilm activity against *Candida* spp. has not been exhaustively tested.

4. The future

Drug discovery and development represents an arduous, failure-prone, time-consuming and very costly proposition; up to 20 years and 2 billion dollars [49]. Thus, as we look towards the future of *Candida* biofilm treatment, we need to consider current work being performed today in a number of, mostly, academic laboratories which could serve as the basis for future management strategies. This work is now firmly based on our increasing understanding of *Candida* biofilm biology, physiology, resistance and pathogenesis; and greatly facilitated by a number of methodologies developed during the last couple of decades to study *Candida* biofilms [9,50]. Thus, numerous groups around the world are harnessing these existing knowledge and technical skills in order to identify and further develop novel drugs and alternative strategies for the therapy of biofilm-associated *Candida* infections. In this section we would like to highlight what we consider to be some of the most promising and advanced approaches, without discounting many others which may also eventually offer excellent prospects to add or complement our existing antifungal arsenal and management modalities. We would like to note that none of the approaches mentioned below have yet reached the clinical setting.

4.1. Screening of chemical libraries to identify new compounds with inhibitory activity against Candida biofilms

For many years now screening, and particularly high throughput screening (HTS), have become the cornerstone of drug discovery in the pharmaceutical industry. With the advances in technology, the existence of high quality and diverse chemical libraries, as well as the establishment of core screening facilities in a number of universities, academic laboratories can now implement these techniques in search of inhibitors of their particular process of interest. Low and medium throughput screening techniques can also be implemented in more resource-limited laboratories even in the absence of sophisticated equipment. The main attractiveness of these techniques is the savings in cost, time, reagents and effort involved in the identification of novel chemical matter of interest.

To illustrate this approach, readers are referred to two relatively recent articles on the screening, identification and further characterization of small molecule inhibitors of *C. albicans* biofilm formation, for which a total of 50,000 compounds from commercially available chemical libraries were screened using the well established multi-well format of *Candida* biofilm formation and susceptibility testing. Pierce et al. reported on the identification of a new series of diazaspiro-decane structural analogs which were largely represented among the bioactive compounds [51]; whereas as reported in Romo et al. the two main hits belonged to a novel series of bioactive compounds with a common biaryl amide core structure [52–54]. After a series of dose-response secondary assays to confirm their biofilm inhibitory activity, establishing their potency, and determining their cytotoxicity, the leading compounds in these studies underwent subsequent in vitro and in vivo characterization. To briefly summarize some of the main observations: i) both leading compounds inhibited *C. albicans* biofilm formation and filamentation in vitro without affecting planktonic growth at relatively low concentrations (approximately 5 micromolar), ii) serial passage experiments indicated that prolonged exposure to increasing concentrations of these compounds are highly unlikely to induce resistance, iii) both compounds were effective in vivo in the murine models of hematogenously disseminated and oral candidiasis [51–54]. Altogether these biofilm inhibitors, with novel chemical structures and mode of action (inhibition of biofilm formation), behave as true anti-virulence compounds; and these studies provide proof of concept for the future implementation of anti-virulence approaches against *C. albicans* (and potentially other fungal infections) which would be less likely to foster the development of resistance.

Also, a complementary approach has been the screening of chemical libraries in search for compounds that synergize with or potentiate the anti-*Candida* biofilm activity of current, clinically-used antifungal agents. One such screen was reported by the LaFleur group, to identify potentiators of the clotrimazole biofilm activity. Using HTS, they identified a total of 19 potentiators of clotrimazole biofilm activity against *C. albicans*, which were subsequently validated for their ability to inhibit biofilms alone, and in the presence of clotrimazole [55]. Likewise, the Thevissen group identified artemisinins as new potentiators of miconazole activity against *C. albicans* biofilms [56].

4.2. Turbinmicin

The Andes group recently reported on the discovery of a novel antifungal molecule, termed turbinmicin, which interestingly is produced by the associated microbiome of a marine animal [57]. The fungal vesicle delivery pathway was identified as the target of turbinmicin. The authors hypothesized and subsequently demonstrated that this new

antifungal inhibited the vesicle-delivered biofilm matrix, thereby effectively negating the protection that the biofilm matrix affords to *C. albicans* biofilms, since many of the *Candida* biofilm matrix components are delivered by extracellular vesicles and this process is critical for drug resistance/tolerance [58]. Some very elegant follow-up studies demonstrated that turbinmicin treatment nearly completely abrogated the production of biofilm vesicles and that this new molecule was also active against other *Candida* spp, including *C. tropicalis*, *C. glabrata*, and *C. auris* [58]. Furthermore, turbinmicin was effective in vivo in the rat central venous catheter model of *C. albicans* biofilms [58], which mimics a severe clinical biofilm infection, thereby corroborating the potential clinical value of turbinmicin as a novel *Candida* biofilm therapeutic.

4.3. EntV

As commensal of humans and also during infection, *Candida* spp interact with multiple bacteria within the normal microbiota. Thus, it is not surprising that some bacteria may actually synergize with or compete and antagonize against *Candida*. Characterization of these interactions may lead to new avenues for the treatment of candidiasis. This is exemplified by a series of articles in the last few years by the Lorenz and Garsin groups, who initially reported on the interactions between *Enterococcus faecalis* and *C. albicans*, whereby the bacterium can inhibit *C. albicans* morphogenesis, biofilm formation, and overall virulence [59]. This effect was associated with a signaling event in which a bacterial-derived product inhibited *C. albicans* filamentation and biofilm formation, with subsequent studies identifying this product as EntV, a bacteriocin produced by *E. faecalis* as a pre-pro-peptide. The active form of EntV is 68 amino acids [60], which per se does not exhibit antifungal activity, inhibits *C. albicans* biofilm formation both in vitro and in vivo [61]. In a recent study aimed at optimizing its potential anti-*Candida* therapeutic the authors identified a shorter 12-mer peptide derived from EntV which maintained the inhibitory activity, including against *Candida* biofilms [62].

4.4. Repurposing

Drug repurposing, also referred to as repositioning, is the search for new therapeutic indications for already existing drugs [63,64], which constitutes an auspicious alternative pathway to antifungal drug development [65]. This pathway is particularly appealing within the academic environment and offers an ideal opportunity for collaboration between academia, governments, and international organizations, to fill the existing void in the antifungal drug pipeline. One of the distinct advantages of repurposing is the fact that the leading candidate repositionable drugs are already approved or at the very least have been through several stages of clinical development, and therefore the pharmacological properties of these drugs, and their safety in humans are already established [63,64]. This makes the repurposing pathway significantly faster, cheaper, and more likely to succeed as compared to "de novo" drug discovery that searches for entirely new chemical matter. As such, it can also lead to a much faster deployment of new antifungals and significantly shorten the translation from the bench to the bedside [66,67].

There are many notable examples of repurposing efforts by many different groups of investigators in the antifungal space, and readers are referred to excellent review on this topic. Initial repurposing efforts were mostly piece-meal, focusing on single or perhaps a handful of compounds in order to identify their potential antifungal activity; but more recent efforts have adopted the more powerful "screening" strategy from the drug discovery field, as described above [68]. These have also benefited from the availability of a number of "repurposing libraries" where hundreds to thousands of drugs can be screened at a fast pace using relevant models of *Candida* growth and/or biofilm formation. There are many examples of such efforts in the recent literature aimed specifically at the identification of repositionable compounds with anti-*Candida* biofilm inhibitory activity [68]. While initial studies mostly used *C. albicans*, most recently these studies have been also extended to *C. auris*, due to the urgency in identifying new compounds effective against this multi-drug resistant emergent species [67,69,70]. A few notable leading repositionable candidates, among others, identified during this work are auranofin, ebselen, alexidine and niclosamide, with ongoing studies aimed at further evaluating their activity both in vitro and in vivo, including in biofilm-relevant models, and advancing their development as antifungals, with emphasis on the treatment of resistant *Candida* infections, including biofilm-associated candidiasis [69,71–74].

4.5. Nanotechnological approaches

Nanotechnological approaches represent another promising alternative for the prevention and treatment of biofilm-associated infections, which has been gaining traction over the last few years in the field of *Candida* biofilms [75]. Generally speaking, "nanomaterials", "nanoparticles" or "nanoantibiotics" are considered to be single-structures, free or in a composite, with a size of less than 100 nm in at least one of their three dimensions. Within this nanometric scale the physicochemical properties of materials display new or improved physicochemical properties as compared to the same materials at larger scales. The inhibitory activity of a variety of nanomaterials against *Candida* biofilms has been evaluated during approximately the last 10–15 years, with the majority of research being on metal nanoparticles, and more specifically silver nanoparticles (AgNPs), synthesized by different methods. While initial experiments demonstrated the increased activity of AgNPs against *C. albicans*, including preformed biofilms [76,77], most recently this research has also expanded to other *Candida* spp., and in particular *C. auris* [78,79]. The increased antifungal activity of nanoparticles is generally associated with the smaller size of the nanoparticles, with shape and surface area also playing a major role. Ultrastructural observations indicated that the anti-biofilm effect of AgNPs is achieved mostly via cell wall disruption of *Candida* cells [76]. AgNPs can also accumulate outside the *Candida* cell surface, interact with cell wall components and in the process release ionic silver leading to cell death [80]. The synthesis and anti-*Candida* biofilm activity of bismuth metallic nanoparticles has also been recently reported [81–83]. Other types of nanoparticles, including different polymeric nanoparticles (*i.e.* chitosan, curcumin) also display inhibitory activity against *Candida* biofilms [84,85]. Importantly, nanoparticles have been described to display potent activity against mixed *Candida*/bacterial biofilms which normally exhibit high levels of resistance against both antibacterial and antifungal antibiotics [86]. Of course, one of the current major impediments of the use of nanoparticles is their limitations for systemic therapy.

4.6. Other approaches

Due to the multiple alternative strategies being developed early in the "basic" research process, the following list is not meant to be comprehensive, but rather our intention is to highlight just a handful of other approaches currently under investigation which we believe hold promise for their eventual utilization to combat the threat of *Candida* biofilms. These include the use antimicrobial peptides, inhibitors of other components within the biofilm matrix (*i.e.* extracellular DNA), the use of probiotics, hsp90 inhibition, as well as modulators of quorum sensing [87–93]. An interesting methodology being developed and applied to the *Candida* biofilm field is that of photodynamic therapy (PDT), where the inhibitory activity is mediated by the action of reactive oxygen species generated by the photoactivation of a photosensitizer by a light source [94–97]. For catheter-related candidemia, several promising strategies are the development of catheter locks [98,99], the development of novel surface coatings which inhibit *Candida* attachment and/or subsequent biofilm formation [100,101], and the

Table 1
Novel approaches against *Candida* biofilms.

Approach	Main thrust/techniques	Representative references
Screening Chemical Libraries	Identification of new compounds with anti-biofilm activity, alone or in combination High throughput screening (HTS)	51, 52, 55, 56
Turbinmicin	Produced by the microbiome of a marine animal Inhibits extracellular vesicle-delivered biofilm matrix	57, 58
EntV	Antifungal peptide produced by *E. faecalis*	60
Drug Repurposing	Search for new therapeutic indications for existing drugs Screening Repurposing libraries	68
Nanotechnology	Nanomaterials, nanoparticles or nanoantibiotics Size of less than 100 nm in at least one of their three dimensions Potent anti-biofilm activity, including against mixed biofilms	76, 78
Other[a]	Antimicrobial peptides Targeting extracellular DNA Probiotics Hsp 90 inhibition Modulators of quorum-sensing Photodynamic therapy (PDT) Catheter locks Surface coatings Inhibition of dispersion	87–102

[a] this list is not all inclusive.

inhibition of biofilm dispersal from a catheter biofilm that would prevent the most serious establishment of invasive candidiasis at distal organs [102] (Table 1).

5. Conclusion

There are significant shortcomings associated with the management of *Candida* infections with a biofilm etiology. Notably, the lack of effective antifungal therapeutics greatly contributes to the excess morbidity and mortality rates associated with biofilm-associated candidiasis. Research on *Candida* biofilms has literally exploded in the last two decades. Lessons from the past and our increasing understanding of *Candida* biofilms offer new opportunities for the development of novel therapeutics to combat the threat that these devastating infections pose to an increasing number of at-risk patients.

CRediT authorship contribution statement

Olabayo H. Ajetunmobi: Writing – original draft, Preparation. **Hamid Badali:** Writing – original draft, Preparation. **Jesus A. Romo:** Writing – original draft, Preparation. **Gordon Ramage:** Writing – original draft, Preparation, Writing – review & editing. **Jose L. Lopez-Ribot:** Writing – original draft, Preparation, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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