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COMMENTARY

Candida albicans and Antifungal Peptides

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ABSTRACT

Candida albicans, a ubiquitous opportunistic fungal pathogen, plays a pivotal role in human health and disease. As a commensal organism, it normally resides harmlessly within the human microbiota. However, under certain conditions, *C. albicans* can transition into a pathogenic state, leading to various infections collectively known as candidiasis. With the increasing prevalence of immunocompromised individuals and the widespread use of invasive medical procedures, candidiasis has become a significant public health concern. The emergence of drug-resistant strains further complicates treatment options, highlighting the urgent need for alternative therapeutic strategies. Antifungal peptides (AFPs) have gained considerable attention as potential candidates for combating *Candida* spp. infections. These naturally occurring peptides possess broad-spectrum antimicrobial activity, including specific efficacy against *C. albicans*. AFPs exhibit several advantageous properties, such as rapid killing kinetics, low propensity for resistance development, and diverse mechanisms of action, making them promising alternatives to conventional antifungal agents. In recent years, extensive research has focused on discovering and developing novel AFPs with improved efficacy and selectivity against *Candida* species. Advances in biotechnology and synthetic peptide design have enabled the modification and optimization of natural peptides, enhancing their stability, bioavailability, and therapeutic potential. Nevertheless, several challenges must be addressed before AFPs can be widely implemented in clinical practice. These include optimizing peptide stability, enhancing delivery methods, overcoming potential toxicity concerns, and conducting comprehensive preclinical and clinical studies. This commentary presents a short overview of candidemia and AFP; articles and reviews published in the last 10 years were searched on The National Library of Medicine (National Center for Biotechnology Information–NIH–PubMed). The terms used were *C. albicans* infections, antimicrobial peptides, antifungal peptides, antifungal peptides mechanisms of action, candidemia treatments and guidelines, synthetic peptides and their challenges, and antimicrobial peptides in clinical trials as the main ones. Older publications were cited if they brought some relevant concept or helped to bring a perspective into our

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narrative. Articles older than 20 years and those that appeared in PubMed but did not match our goal to bring updated information about using antifungal peptides as an alternative to *C. albicans* infections were not considered.

Keywords: Antifungal; Peptides; Candida infections; Candidemia

Key Summary Points

Why carry out this study?

This article aims to provide an overview of antimicrobial peptides (AMPs), their mechanisms of action, and their potential applications in combating microbial infections

AMPs are naturally occurring small polypeptides found in various organisms and have characteristics, including positive charges and hydrophobic/hydrophilic residues, that allow them to target microbial membranes with reduced toxicity to mammalian cells

AMPs against fungi are called antifungal peptides (AFP), and this article provides an overview of AFPs, their mechanisms of action, and their potential applications in combating fungemia, especially against *Candida albicans*

What was learned from the study?

Some AFPs presented immunomodulatory responses by affecting cytokine production and immune cell activation, which can be further exploited as a possible therapeutic approach

There are challenges associated with using AFPs as therapeutics. Issues to overcome include susceptibility to proteolytic degradation, interactions with ions, and toxicity, which researchers are addressing through protein engineering and other strategies

INTRODUCTION

Members of the genus *Candida* are heterogeneous, being able to grow both as yeasts and in the more elongated yeast forms characterized by pseudohypha and pseudo-mycelium. It should be noted that only *Candida albicans* and *Candida dubliniensis* can form true hyphae (true mycelium), these two species being considered polymorphic [1]. There are about 150 species of *Candida* spp., but only a small number have been considered human pathogens. These yeasts are part of the normal human microbiota and are found in mucous membranes (30–60%), gastrointestinal and genitourinary tracts of healthy people, and the yeast–host interaction occurs throughout life. Thus, it is possible that a person can encounter yeasts several times a day without major physiological complications [2–4]. Alterations in host defenses, however, constitute factors that may favor systemic invasion by *Candida* spp., representing the main agents involved in nosocomial infections with high mortality rates, especially in developing countries [5].

The epithelium and mucous membranes of the body constitute the primary barrier to preventing microorganism entry. Invasive infections happen when ruptures in these barriers occur and may be physical or associated with decreased immune response [6–8]. Fungi of the genus *Candida* can invade virtually all body parts, including superficial and deep organs [9]. Evidence suggests that tissue colonization can occur by hyphae in the endothelium and by the passage of yeast-like forms to adjacent tissues. Thus, the polymorphism of this microorganism is essential for the colonization and persistence of the infection [10, 11]. Hyphae formation is also a way for the fungus to adapt to the adverse conditions of different environments and their particularities in the same host [12, 13]. Among the environmental stimuli that favor the change from yeast to hypha we can mention the presence of serum at a temperature of 37 °C, neutral pH, 5% of carbon dioxide, and microaerophilic conditions [14, 15]. These stimuli can

activate cAMP/PKA (cyclic 3′,5′-adenosine-monophosphate/protein kinase A) or MAPK (mitogen activated protein kinase), leading to the activation and expression of genes related to hyphal formation, such as the transcription factors Efg1 (related to metabolic adaptions and morphogenesis) and Cph1, activation of the cell wall gene Hwp1 (hyphal wall protein 1), the agglutinin-like protein sequence Als3 (agglutinin-like sequence protein 3), and the secreted aspartic proteases (Sap) Sap4, Sap5, and Sap6, as well as Ece1, and Hyr1 hyphal-associated proteins [16–18]. Once formed, the hyphae produce other agglutinins (Als) by expressing the ALS gene (ALS1–7 and ALS9), which are considered surface proteins, and glycosylphosphatidylinositol (GPI) linked to β-1,3-glycan of the fungal cell wall [19, 20]. The colonized tissue can serve as a source of dissemination of yeasts through the bloodstream. This process can occur in two ways already described: from the induction of endocytosis by host cells and the fungus's active penetration. The first is an actin-dependent process in which the interaction of host receptors with fungal adhesins, such as Als3, stimulates endocytosis [16]. Active penetration, conversely, includes elongation of the hypha by intercellular junctions, exerting mechanical pressure in addition to the secretion of hydrolytic enzymes. Saps help in the degradation of E-cadherin, present in intercellular junctions, leading to the loss of endothelial integrity, a process aided by the secretion of mucins and phospholipases [21, 22].

Hematogenous dissemination of *C. albicans* can occur in immunosuppressed patients, especially in cases of neutropenia and those with critical clinical conditions [23]. In these patients, the source of contamination or persistence of infection may be associated with the formation of biofilms on medical devices, especially catheters, whose colonization can occur endogenously or exogenously from the hospital environment [24, 25]. Other risk factors are patients admitted to the intensive care unit (ICU) and those who undergo extensive abdominal surgeries or have renal failure, patients on hemodialysis or using broad-spectrum antibiotics, corticosteroids, and total parenteral nutrition [26, 27].

Invasive candidiasis, also known as systemic candidiasis, candidemias, or hematogenous disseminated candidiasis, involves the spread of *Candida* spp. via the bloodstream to multiple organs: the brain, kidneys, heart, lungs, and liver [28, 29]. Overall, 40–60% of patients who develop candidemia fail to progress to cure, with high mortality due to late diagnosis and severity of comorbidities. Candidemia can also be frequent in neonates, whose immunity is not fully developed, making this group susceptible to a wide range of pathogens [30]. Another common manifestation of colonization or infection by *Candida* spp. in hospitalized patients is the presence of candiduria, defined by the presence of yeasts in the urine, which may indicate infectious processes in the kidneys, with high morbidity and mortality [31].

The success of infection and persistence in organisms is also related to fungal mechanisms capable of evading the host's immune system. Among the virulence factors of *C. albicans* is the ability to adhere to host cells and to secrete degrading enzymes. These factors and host predispositions help to set up candidemia [22, 32]. The mechanism of action of each virulence factor and the sequence of events that culminate in systemic infection are still at the hypothesis stage [7]. Adhesion to host cells is related to the existence of molecules called adhesins (polysaccharides, glycoproteins, and lipids). Evidence suggests that adhesins guide the growth of hyphae along the grooves of tissue surfaces or devices that are in the patient's body, such as catheters [33, 34]. Evidence also suggests that heat shock proteins are associated with the virulence process. The heat shock transcription factor 1 (Hsf1) and the heat shock chaperone Hsp90 coordinate the conformational change of chromatin and the expression of stress-associated genes, allowing an adaptation to the elevation of host temperature (fever), and participating in metabolic pathways that result in cell wall composition [35, 36]. The candidalysin peptide originates in the proteolytic processing of the protein originating from the ECE1 gene, processed by Kex2/Kex1, being just one of the eight potential peptides generated by Kex2. Candidalysin is also a virulence factor secreted by the invasive form of

C. albicans, which can damage the membrane of host cells. Yeasts of *C. albicans* which express ECE1 tend to form hyphae for tissue adhesion, but without tissue damage or induction of a response to stimulation of the MAPK pathway [37, 38].

Patients with candidemia have a positive blood culture for *Candida* spp., and usually show signs of ongoing systemic infection, such as fever, hypotension, and tachycardia [39]. Patients with acute candidemia are considered if there is evidence of involvement of multiple non-contiguous organs. The colonization and infection of the urinary system is another possible indication of candidemia when the agent is isolated in urine culture [40–42]. Other possible clinical manifestations in fungemia include pyelonephritis, peritonitis, arthritis, hepatosplenic abscesses, myositis, macronodular lesions in the dermis, endophthalmitis, meningitis, and generalized systemic involvement [43, 44]. These clinical signs, however, may be related to other fungal and bacterial diseases; in cases of sepsis, the diagnosis and isolation of the infectious agent must therefore be performed with greater precision and in the shortest possible time [45, 46].

Blood culture is still considered the gold standard for diagnosing candidemia, and automated systems have been advocated for allowing monitoring of cultures every 10 min by colorimetric reactions in the medium (BacT/ALERT 3D, Organon Te Kinika Corp. Durham, NC), or by fluorescence (BACTEC 9240, Becton Dickinson, USA) [47]. Immunodiagnostic tests allow the detection of specific antibodies or fungal structures, such as anti-mannan antibodies, and immunofluorescent assays are used for detecting antibodies against germ tubes. Enzyme linked immunosorbent assays (ELISA) are used to detect antigens such as α-linked oligomannose residues, which make up 7% of the composition of the fungal wall, as well as 1,3-β-D-glucan, a polysaccharide also present in the fungi cell wall. Molecular diagnostic techniques include polymerase chain reaction (PCR) [48–50].

Candidemia can be treated with polyene antifungals (amphotericin B) and its derivative lipid formulations, azoles (fluconazole, itraconazole, voriconazole, posaconazole), and echinocandins (caspofungin, micafungin, and anidulafungin). Amphotericin B was once considered the gold standard for candidemia and other invasive fungal infections. However, its use has become more limited because of nephrotoxicity and renal failure (49–65% of treated patients) as its most important side effects. Their lipid formulations have the same efficiency and less toxic side effects but with higher costs. Among the drugs available for treatment, fluconazole has the best oral absorption (close to 100%). It is not affected by gastric pH [51–53]. Its preferential use in the treatment of candidemia is also because fluconazole can concentrate in the urine, allowing the treatment of urinary infections caused by *Candida* spp. [54]. Echinocandins are the newest class of antifungals and act by inhibiting β-D-glucan synthase, thereby interfering in the synthesis of the fungal cell wall; echinocandins have high efficiency in invasive infections and are recommended as initial therapy based on high-quality evidence of an association with reduced mortality from patient-level analysis of randomized trial data [50, 55, 56]. Itraconazole, on the other hand, has limited use due to its low bioavailability when administered orally, with a high incidence of adverse effects and drug interactions [57, 58].

The increase in the incidence of candidemia is related to the increase in at-risk population, the performance of invasive procedures and the application of medical devices for continuous use (catheters), and the indiscriminate use of broad-spectrum antibiotics, in addition to the formulations used in chemotherapy [59]. Inappropriate antifungal therapy within 72 h and ICU admission are also associated with mortality in candidemia [60]. Among the control measures proposed to minimize the occurrence of these fungemias in hospital environments are an attempt to maintain patients only for the strictly necessary period, especially in ICUs, and promoting hygiene education aimed at patients, visitors, and health maintenance teams because *Candida* spp. can survive up to 45 min on hands after contamination, and can be transmitted by contact to contaminated surfaces [61, 62]. Additionally, the maintenance

time of catheters and their management, as well as other medical devices, is crucial in the success of therapy against persistent candidemia. Evidence also suggests that candidemia is associated with therapeutic failures and emerging resistance to polyene and azole antifungals [50, 63].

Candida spp. accounts for 80% of nosocomial fungal infections [64]. Candidemia is the most common cause of hematogenous infections in the USA and the most frequent in patients infected during ICU treatment in many parts of the world [65]. According to Soulountsi et al. [66], 70–90% of fungal infections in ICU are invasive candidiasis, and 5–15% of patients are colonized by *Candida* spp. on ICU admission. The proportion of patients with candidemia who develop sepsis and septic shock from fungi is 8–30% and 23–38%, respectively. An increased length of ICU stay of 10.1 days was associated with a 14.5% increase in mortality, which was observed in a US epidemiological study with 30 cases per 100,000 admissions as the incidence of candidiasis [67, 68]. According to Pendrak et al. [69], two out of three cases of systemic *Candida* spp. infection are of hospital origin and the estimate of the total cases in the USA is 10,500–42,000. Furthermore, the mortality rate was estimated to be between 46% and 75% and there was high morbidity in patients who survived the infection. In patients who underwent liver transplantation, the mortality rate from *Candida* spp. was greater than 60% [7, 69, 70]. According to the North American program for research and control of pathogens of epidemiological importance (Surveillance and Control of Pathogens of Epidemiological Importance—SCOPE), of the 24,179 cases of systemic fungal infections diagnosed in 49 North American hospitals between the years 1995 and 2002, 4.6 per 10,000 cases were attributed to *C. albicans*, accounting for 9% of cases of hematogenous disseminated infections [70–72].

Despite medical advancements, the incidence of invasive fungal diseases is continuously on the rise [73, 74]. This concerning trend can be further aggravated by the expansion of endemic fungi and the emergence of new pathogens, both of which are consequences of climate change [75, 76]. The battle against fungal diseases poses a significant challenge due to the nature of the pathogens and the limited arsenal of antifungal drugs. The current repertoire of antifungal medications remains relatively narrow, with representative drug classes including azoles, polyenes, echinocandins, and pyrimidine analogues. However, these drugs often encounter fungal resistance and notable off-target effects [77, 78].

AMPs are a promising tool to enhance the drug arsenal against various microorganisms. Mechanisms of action, immunomodulatory activities, and use of protein engineering are briefly presented in the following text, as well as antifungal peptides (AFPs) against *C. albicans* and clinical trials and their challenges.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

ANTIMICROBIAL PEPTIDES (AMPS)

AMPs are small polypeptides (mostly positive charged, + 2 to + 7) with 2–50 amino acid residues isolated from virtually all life forms. Biologically, they are synthesized mainly in ribosomes and may undergo post-translational modifications, with current techniques of protein synthesis being used for the large-scale production of synthetic peptides [79]. AMPs are part of the organism's innate immune response. Evidence suggests that the gene sequences that code for their syntheses have remained conserved throughout the evolution of organisms, with constitutive expression at basal levels and whose transcription possibly occurs as a result of contact or exposure to pathogens [80]. They generally have a broad spectrum of action against bacteria, viruses, and fungi. Some of the characteristics of these molecules corroborate their activity because they have a net charge, which is primarily positive, and have both hydrophobic and hydrophilic residues, which allow their solubilization in an aqueous medium and with the possibility of crossing hydrophobic membranes. Another point to be emphasized is that these characteristics

privilege the interaction with the negatively charged membranes of microorganisms instead of the zwitterionic cell envelopes of mammals, thereby reducing the possibility of toxic effects of these molecules [81, 82]. The sequence and composition of amino acid residues, as well as the physical–chemical structure (total net charge, structural flexibility, size and percentage of hydrophobicity, and amphipathic characteristics), indicate that they are directly related to the activities of AMPs, especially concerning the interactions of these molecules both with the membranes of microorganisms and with the neutralization or interference in intracellular targets [83, 84]. This characteristic of AMPs confers an advantage since microbial resistance by gene mutation has been less likely [85]. It is also possible that AMPs favor the attraction of charged phospholipids to less thick and more fragile regions of the membrane, making it thinner. AMPs may also favor the oxidation of lipids through the generation of intermediate oxygen species and attract anions that may affect the membrane potential and, consequently, decrease the permeability of this structure to various molecules, thereby hindering cell metabolism [85]. Other forms of action of AMPs involve targets for DNA and protein synthesis, as well as protein folding, enzymatic activity, and cell wall synthesis [86, 87]. It is suggested, therefore, that AMPs significantly interfere with cell metabolism, which may lead to cell death from possible interactions with promoter genes and coding sequences, and they may interfere with enzymatic synthesis and protein folding [82, 83].

AMPs show great promise as molecules effective against various pathogens. The Database for Antimicrobial Activity and Structure of Peptides (DBAASP; https://dbaasp.org) was established in 2014 to serve as a comprehensive collection of published data on AMPs. It has a dual purpose, acting both as a repository for AMP-related literature and as a resource for supporting studies on the relationship between peptide structure and activity. Over time, DBAASP has undergone significant growth and enhancements. DBAASP is widely used in the research community and has contributed to developing other peptide-related databases. It offers predictive tools for designing peptides with desired antimicrobial properties, and its extensively curated data serves as a valuable source for building statistical models. DBAASP lists approximately 18,400 peptides, with 5967 targeting fungi. This specific group of AMPs, known as antifungal peptides (AFPs), includes compounds that combat *Candida* spp., *Aspergillus* spp., and *Cryptococcus neoformans* [88, 89]. Like AMPs, AFPs exhibit their antimicrobial activity by interacting with the fungal membrane, resulting in the formation of pores using a barrel stave model, toroidal pore model, or through accumulation on the membrane until disruption occurs (Fig. 1). AFPs can also interact with intracellular targets, such as nucleic acids and chitins, with unknown mechanisms of action. Usually, AFPs are positively charged and tend to adopt secondary structures when associated with cell membranes. Also, the percentage of hydrophobic residues, length, and amphiphilicity affect their activity [90, 91].

AMPs involved in antimicrobial activity against bacteria and viruses have been widely studied, although therapeutic strategies to control fungal diseases with these molecules have still been limited [79]. The manipulation of the immune system for the development of vaccines against fungi, activation of the specific immune response directed to fungal agents (in the form of targeted stimulation of phagocytes, T cells, and antibodies), as well as the induction of the innate or adaptative immune response, with the manipulation of the profile of cytokines, constitute a challenge for therapeutic innovation, with data in the literature being less frequent than those found for bacteria and viruses [92, 93].

The literature reports around 887 AFPs with described fungicidal activity against *C. albicans*. Among the mechanisms of action, the induction of the formation of reactive oxygen species occurs with the histatin-5 (Hst-5) peptide and lactoferrin-derived peptides [93]. Hst-5 binds *Candida* spp. cell wall proteins (Ssa1/2) and glycans, which are taken by the fungal cell through fungal polyamine transporters. Once inside the cell, Hst-5 affects mitochondrial functions and causes oxidative stress, with volume dysregulation and ion imbalance triggered

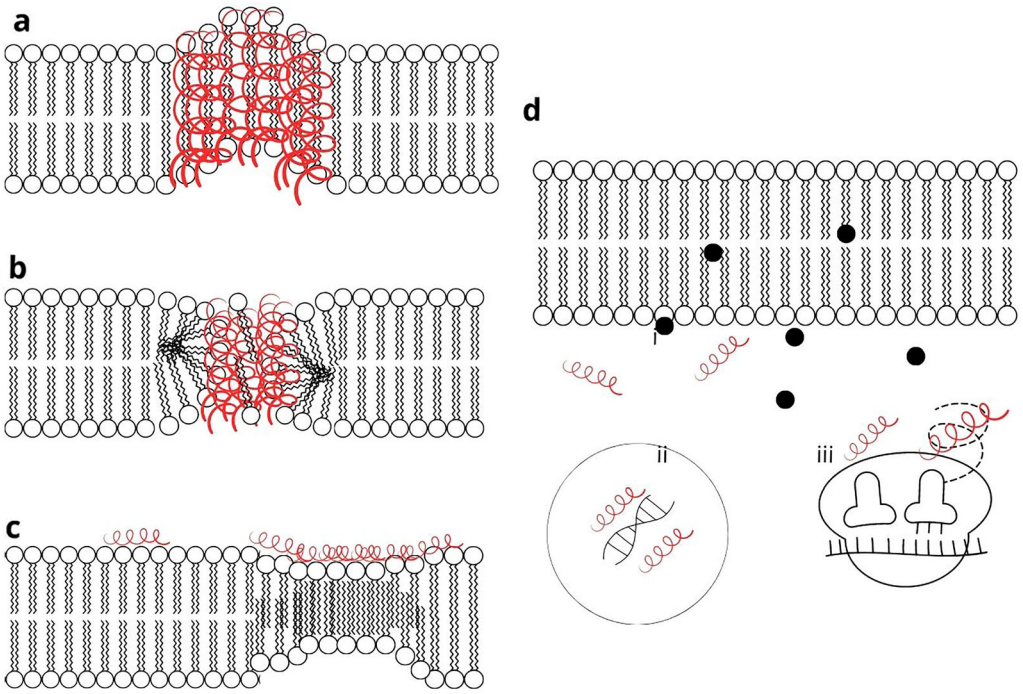

Fig. 1 Possible AFPs and membrane interactions. **a** Pore formation in a barrel stave model; **b** pore formation in a toroidal pore mode; **c** AFP accumulation on the less thick and more fragile regions of the membrane, making it thinner; **d** other possible mechanisms of action: i—lipid oxidation through intermediate oxygen species (black dots); ii—targeting DNA; and iii—interference in protein synthesis. AFPs are generically represented as red coils

by osmotic stress leading to cell death [94]. Depletion of mitochondrial activity has also been observed in the peptide tenecin-3, capable of decreasing fungal cell viability when in contact with the cell cytoplasm [95]. Studies show that induction of actin depolarization is caused in fungal cells by a plant defensin called *Phartibis nil* antimicrobial peptide 1 (Pn-AMP1) [96, 97]. Synthetic peptides analogous to the neutrophil-derived CAP37 cationic domain have shown fungicidal activity against isolates of *Candida* spp., specifically *C. albicans*, *C. guillhermondii*, *C. tropicalis*, *C. pseudotropicalis*, *C. parapsilosis*, and *C. dubliniensis* [98]. The AMP dolabellanin B has been isolated from sea slugs *Dolabella auricularia* and showed antifungal activity against *C. tropicalis*, *C. albicans*, and *Saccharomyces cerevisiae* [99].

The use of AMPs in combination with drugs routinely used in therapy has been described, such as the P10 peptide (derived from the gp43 antigen of *Paracoccidioides* spp.) with amphotericin B and azoles (fluconazole, ketoconazole, and itraconazole), which was able to improve the survival of mice with respiratory tract infected with *Paracoccidioides brasiliensis* [100, 101]. Fais et al. [102] reported the synergistic activity of human lactoferrin-derived hLF1-11 peptide and caspofungin, yielding drastic caspofungin minimal inhibitory concentration (MIC) reduction and synergistic inhibition of biofilm formation by biofilm-producing strains of *Candida* spp., restoring sensitivity to caspofungin in caspofungin-resistant strains, both in free-living cells and in biofilms.

In addition to antimicrobial effects, AMPs can act as modulators of the immune response, participating at different cellular levels, interacting directly with immunogenic agents and modulating the inflammatory response [103]. This immunomodulation can occur through cellular processes such as modulation of pro-inflammatory cytokines, inhibition of the inflammatory response, chemotaxis and

recruitment of macrophages, neutrophils, and eosinophils, differentiation of dendritic cells, activation of lymphocytes, presentation of antigens, angiogenesis, and healing of wounds [104–106]. These activities are proposed on the basis of studies and the observation that the concentration of peptides in vivo is lower than the MIC assays performed in vitro. Another proposed explanation is the possible synergistic action with other peptides. An example of this is the LL37 peptide, derived from a cathelicidin hCAP18, with 37 amino acids, whose sequence starts with two lysines and which has a broad spectrum of antimicrobial action. LL37 can act together with lysozyme and lactoferrin to potentiate their microbicidal activity. Peptides can also accumulate locally to reach the MIC concentration necessary to defeat a microorganism [107–109]. The indirect chemotactic activity of AMPs can occur by chemokine stimulation or cytokine secretion by different cell types through receptor-dependent mechanisms, such as what occurs with LL37, which optimizes interleukin (IL)-6 and IL-10 production from IL-1β [107]. Yang et al. [110] suggest that human α- and β-defensins are chemotactic for T cells and dendritic cells, respectively, and with β-defensins they can also stimulate the migration of keratinocytes, helping wound healing [104].

One of the synthetic AMPs that has been investigated for its antimicrobial and immunomodulatory activity based on similarities with innate defense regulators is IDR-1018, a cationic peptide with 12 amino acid residues (VRLIVAVRIWRR), with a molecular mass of 1536.9 Da, and resulting from mutations and deletions of the Bac2A peptide (a linear derivative of a cathelicidin bactenicin found in bovine neutrophils) [111–113]. Pena et al. [113] observed that this peptide induces an intermediate cytokine profile between the stages of differentiation of macrophages M1, with pro-inflammatory characteristics, and M2, with an anti-inflammatory profile and restoration of the plasticity of this group of phagocytic cells. IDR-1018 also increased MCP-1 and IL-10 levels of the bone marrow-derived macrophages cytokine profile challenged with heat-killed *C. albicans* antigens (HKCA), while suppressing tumor necrosis factor alpha (TNFα), IL-1β, IL-6, and IL-12 levels. A murine model of experimental candidemia treated with IDR-1018 showed an increase in the survival rate as well as a diminished kidney fungal burden [114].

It should be noted that the active or passive involvement of the host's immune system and the use of antimicrobial drugs is a preponderant factor in the response to infectious agents. Some of the plausible justifications are the fact that the reduction in the number of pathogens allows the immune system to clean up dead cells, help reduce the remaining pathogens and process deleterious metabolites excreted both by microorganisms and their death (such as the mycotoxins) and collateral tissue damage generated during the inflammatory process [115].

Despite the strategies used by different compounds to defeat microorganisms, they have mechanisms to adapt to drugs to which they were already susceptible, generating the phenomenon known as microbial resistance [53]. The use of AMPs is a promising therapeutic alternative. However, the spectrum of antimicrobial resistance to AMPs cannot be ruled out, even though it is less frequently described when compared to resistance found for drugs routinely used in medical practice. There are reports of bacteria that can become resistant to AMPs as a result of intrinsic characteristics or acquired by microorganisms [116]. The first can occur because of adaptation to the presence of AMPs in the environment in which the microorganisms are found or to the resistance induced by molecular modifications of the possible targets of AMPs, making them less susceptible. Microorganisms may be able to change the lipid composition of the cell membrane, in addition to the possible production of enzymes that can act in the proteolytic cleavage of AMPs [101, 117].

Systemic use of peptides has been an essential barrier to commercialization, especially because peptides are susceptible to proteolytic degradation, and interactions with ions and salt present in body fluids decrease the activity of AFPs and shorten their half-lives [118, 119]. Among the challenges of using AMPs are the possible loss of microbicidal activity of these molecules in the presence of biological

concentrations of cations (Na^{+}, $Mg^{2,+}$ and Ca^{2+}); it is proposed that, in certain situations, the immunomodulatory properties of the peptides are as essential as the antimicrobial activity in fighting infections in vivo [120]. Proteases are present in several physiological processes, and their performance can significantly reduce, or even inactivate, AMPs, which are subject to degradation by proteolytic enzymes present in the blood and elsewhere in the host [121, 122]. Human proteases and those produced by fungal pathogens are important in this process of AFP inactivation, e.g., *C. albicans* secreted aspartic proteases which can inactivate histatin-5 [123, 124]. Another challenge is toxicity, once AFPs need to be selective toward fungal cells over mammalian cells, and those with pore formation as a mechanism of action can be nonspecific once fungal and mammalian membranes are similar [119, 125].

A tool to overcome those limitations is protein engineering strategies, because the chemical synthesis of AFPs is expensive at large scale, and purification in natural sources does not fulfill the market needs. Rational design is based on knowledge of a peptide's structure or function, which can improve antifungal activity and proteolytic and thermal stability [79], by introducing enantiomeric peptides and substitutions by amidated bridges [126, 127]. Other ways of protecting the AMPs involve modifications in the N- and C-terminal portions, as well as cyclic forms aiming to increase the stability of the peptides against physiological salt concentrations and the possibility of degradation by proteases [128–130].

Despite the high costs of isolation or chemical synthesis of AMPs, these molecules have aroused the interest of the pharmaceutical industry. Currently, peptides encounter difficulties in clinical trials due to their high susceptibility to degradation. However, strategies are being studied to overcome these difficulties, including nanotechnology and controlled drug delivery systems [131–133]. Examples of antifungal peptides currently on the market are echinocandins (lipopolypeptides), polymyxin B sulfate, used in infections by multidrug-resistant Gram-negative bacteria, and enfuvirtide (Fuzeon®), used in the treatment of AIDS [134–136].

Currently, about 80 peptides are commercially available in the USA. It is estimated that about 500 peptides are intended for clinical development and another 400–600 are in preclinical studies. A peptide becomes a good candidate for clinical trials and future commercialization if it has similar or better efficacy than drugs available on the market, as well as being tolerable with pharmacodynamics and pharmacokinetics that allow for fewer side effects and safe use in different presentations [137]. Possible production and marketing costs are also considered, so that about 90% of candidates for new therapies fail to meet all these requirements [138, 139]. The use of new technologies that allow the screening of peptide drugs with therapeutic potential enabled a 20% increase in peptides entering clinical phases, with more than 30% of those that enter clinical phase I targeting pain relief or with anticancer activity, and cardiovascular diseases; peptides for combating cancer dominate the phase II (15%) and phase III (40%) clinical trials, with the other peptides aimed at infectious and allergic diseases [140, 141]. Among the peptides currently in the clinical phase are Novexatin® (NP213), a synthetic cyclic peptide developed with positive results in phase IIb clinical trials for fungal nail infections via lysis of the fungal outer membrane; Omiganan (MBI-226; derived from bovine neutrophil indolicidin) for seborrheic dermatitis in phase II; PAC 113, a histatin derivative used as a mouth rinse for oral candidiasis; CZEN-002, for vulvovaginal candidiasis, with positive results in phase IIb [142, 143]; pexiganan (magainin) derived from *Xenopus laevis*, for the treatment of bacterial infections and foot ulcers caused by diabetes, and iseganan (IB-367), derived from porcine leukocytes for the treatment of stomatitis, both in phase I/II, but which were initially ignored because they present the same efficacy as drugs already available on the market for the same uses initially proposed for these peptides [144, 145]. Anti-*Candida* antifungal peptides in preclinical and clinical trials are extensively reviewed by Rodriguez-Castano et al. [79]. Antimicrobial peptides approved for clinical application and

Table 1 AFPs in clinical trials

AFP	Mechanism of action	Pretended use	Effects in clinical experiments	References
Novexatin (NP213)	Fungicidal in a water-based topical formulation that effectively penetrates human nails. It is a highly cationic peptide, lysing fungal outer membrane. NP213 is also assessed for the treatment of catheter infections, genitals warts acne vulgaris, and atopic dermatitis	Fungal nail infection, phase IIb (lysing fungal outer membrane)	43.3% of patients have no fungi detectable by culture of fragments from NP213-treated nails after 180 days, when NP213 was applied daily for 28 days. (ClinicalTrials.gov Identifiers NCT02343627; NCT02933879 and EudraCT No. 2008-001496-29, NCT02343627, NCT00321153, NCT03091426, NCT01784133)	[146, 147]
Omiganan (MBI-226)	Binds to mannan and partially to chitin or glucan. Disrupts the lipid bilayer via a toroidal pore mechanism and changes the organization of the membrane by reducing the levels of glucan and mannan in the cell wall, exposing 1,3-β-D-glucan, and creating ergosterol-dense and ergosterol-free areas. This phase separation of the membrane ultimately leads to membrane permeabilization	Facial seborrheic dermatitis in phase II	Omiganan was safe and well tolerated but did not result in a significant clinical improvement of seborrheic dermatitis when compared to the use of ketoconazole. (ClinicalTrials.gov Identifier NCT03688971)	[148]
PAC113	Targets the mitochondrial complex I, increasing free radicals and inhibiting cellular respiration, with a membrane-lytic activity associated	Histatin-derived used as a mouth rinse for oral candidiasis	Clinical trial ended in 2008, with no results published (ClinicalTrials.gov Identifier NCT0065997)	[90, 91]

Table 1 continued

AFP	Mechanism of action	Pretended use	Effects in clinical experiments	References
CZEN-002	Derived from a melanocyte-stimulating hormone and works as a membrane disruptive agent, as well as disruption of cAMP signaling pathways and immunomodulation, such as the suppression of TNFα production	Vulvovaginal candidiasis, phase IIb	Relief from vaginal candidiasis with positive results: a phase I/II clinical trial reported 88.2% and 87.5% cure, but no follow-up trial (https://www.eurekalert.org/news-releases/796451—accessed October 2023)	[143, 147, 149, 150]

food storage are gramicidin D, gramicidin S, bacitracin, those for topical use against bacteria and also fungi (gramicidin S); nisin (as food preservative against Gram-positive bacteria); polymyxin B, colistin and daptomycin for intravenous use against Gram-negative and Gram-positive bacteria, all of them causing disruption of bacterial membrane as a mechanism of action [141, 143] (Table 1).

AFPs have a promising application in future therapy against fungal infections, especially those caused by *Candida* spp. Like AMPs, AFPs have a diverse mechanism of action, ranging from interactions with cell membranes to intracellular targets, as well as a role in immunomodulation. This article underscores the significant potential of AMPs, notably AFPs, as innovative alternatives to traditional antifungal medications, particularly in response to the growing concern surrounding drug-resistant fungal strains and the limitations of current therapeutic approaches. Moreover, it also discusses the challenges associated with utilizing AMPs, spanning challenges like proteolytic degradation, and ion interactions. With the prospect of AMP/AFP commercialization on the horizon, including those in various stages of clinical development, ongoing research and advances in biotechnology for the meticulous assessment and optimization of AMPs/AFPs are of paramount importance to facilitate their effective integration into clinical practice.

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Declarations

Ethical Approval. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Conflict of Interest. Camila Guimarães de Freitas and Maria Sueli Felipe have nothing to disclose.

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