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The Use of *Candida auris* Therapies based on Nanotechnology as Potential Novel Strategy against COVID-19 Infection: A Mini Review

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ABSTRACT

Candida auris, which is one of the causative agents of candidiasis, has been detected in several individuals with immune deficiency worldwide, mainly in different American countries, since 2012. *C. auris* infections are at risk of becoming epidemic because this species shows multi-drug resistance to several antifungal drugs available in the market; thus, since the current public health condition at global scale is threatened by the SARS-CoV-2 pandemic, *C. auris* infections could lead to high mortality rates. Different strategies, such as drug repurposing and the combination of antifungal drugs to other biocide molecules, were developed. However, they are time-limited strategies since drug resistance has increased due to *C. auris* pathologies. As an alternative, the recent development of nanotechnological devices has opened room for the efficient treatment of *C. auris* infections. Most specifically, the biocide effect of nanoparticles combined to/capped with antifungal drugs in different platforms seems to be an affordable technology to stop invasive *C. auris* infections.

Keywords: Candidiasis; Antimicrobials; Nanoparticles; SARS-CoV-2.

Introduction

Genus *Candida* comprises more than 200 different species, although only a few are the causative agent of mammalian pathologies. Most specifically, fungal species *Candida auris* can grow as yeast and cause severe infection in hospitalized or nursing home patients with weakened immune system. It is one of the few species belonging to genus *Candida* that trigger

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candidiasis in humans. *C. auris* infections do not often react to traditional antifungal drugs used to treat *Candida* spp. infections, since it is said to be resistant to most antifungal drugs. This factor makes it very hard to treat *C. auris* infections; fortunately, this kind of pathology is rare in healthy individuals. In addition, *C. auris* is the *Candida* species mostly isolated in SARS-CoV-2-contaminated blood during the COVID-19 pandemic. Given its ability to cause severe invasive infections, it can lead to mortality rate ranging from 35% to 72% depending on its strain and on patients' physiological condition [1].

The current epidemiological state in Americas is a concern associated with this infection type. The first *C. auris* outbreak in Venezuela was observed in March 2012 [2] and, since then, different countries have reported outbreaks. Among them, one finds Colombia, in February 2015. the United States of America, after mid-2015; Canada, in May 2017; Panama, in September 2017; Costa Rica, in June 2019; Mexico, in May 2020 [3]; and Brazil, in December 2020 [4]. According to CDC, *C. auris* infection cases in 2018 increased by 318% in comparison to the mean number of informed cases from 2015 to 2017 [5].

Since the COVID-19 emergence, worldwide outspread of the SARS-CoV-2 virus has been a major challenge for all American health systems, which reached their maximum hospital care capacity. Patients at the highest risk of developing *C. auris* infection in intensive care units were the most affected individuals. Since July 2020, several countries have reported *C. auris* cases, which were overall associated with COVID-19 patients. Among them, one finds Brazil, Colombia, Guatemala, Mexico, Panama, Peru, and the United States of America [5, 6, 7].

An alert about the first *C. auris* infection case in Brazil (Bahia Sate) was issued by the National Health Surveillance Agency (ANVISA *per* its acronym in Portuguese) in December 2020. No additional cases were reported since then. The outbreak inquires subsequently described broad colonization of patients and environmental contamination with *C. auris* [4].

Rossato and Colombo [8] have pointed out that the most recent studies have shown that *C. auris* overall expresses lesser virulence factors than *Candida albicans*. Meanwhile, the trend *C. auris* infection on healthcare resources is exclusive among *Candida* spp. *C. auris* is possibly to promote virulence and pathogenicity components that favor skin settling and habitat persistence.

Among the cited factors, such as C. *auris* genomics, phenotypic traits and tolerance to thermic and osmotic stresses, the significant role played by lytic enzymes in fungal invasiveness is of paramount importance, since they act as target to new drugs against *C. auris*. The ability of *C. auris* isolates to generate lytic enzymes was already evidenced in the literature, although the generation of these enzymes depends on fungal strain origin and biological source. *Candida* spp. pathogenicity in humans is associated with the generation of phospholipases and proteinases, which play relevant role in supporting the access to, and further invasion of, host cells.

Treating acute fungal infections is a hard task to be accomplished nowadays due to limited antifungal drug availability in the market. The currently available antifungals comprise allylamines (terbinafine), azoles (voriconazole, fluconazole), polyenes (nystatin, amphotericin B), nucleosides (5-flucytosine), and echinocandins (anidulafungin, caspofungin, and micafungin), although some of them may show renal or cardiac toxicity depending on the infection site. For example, nikkomycins belong to the nucleoside amide antibiotic family, they are competitive inhibitors of chitin synthase 3, which is an enzyme involved in cell wall synthesis. Nikkomycins are acknowledged as powerful acaricide and insecticide, as well as present antifungal activity [9]. However, Nikkomycin Z tested on 100 genetically diverse *C. auris* strains has shown wide MIC range from 0.125 to >64 mg/L *in-vitro*, although not all strains were inhibited by this drug [10]. The nikkomycin case typically reflects the sanitary crisis associated with *C. auris*.

The small number of therapeutic options and fungal resistance to the most important drug classes are extremely concerning, since they make this kind of infection highly challenging to be treated [11,12].

C. auris has developed a range of molecular drug-resistance mechanisms, as shown in **Figure 1**, which also present several aspects of such a resistance (**Table 1**).

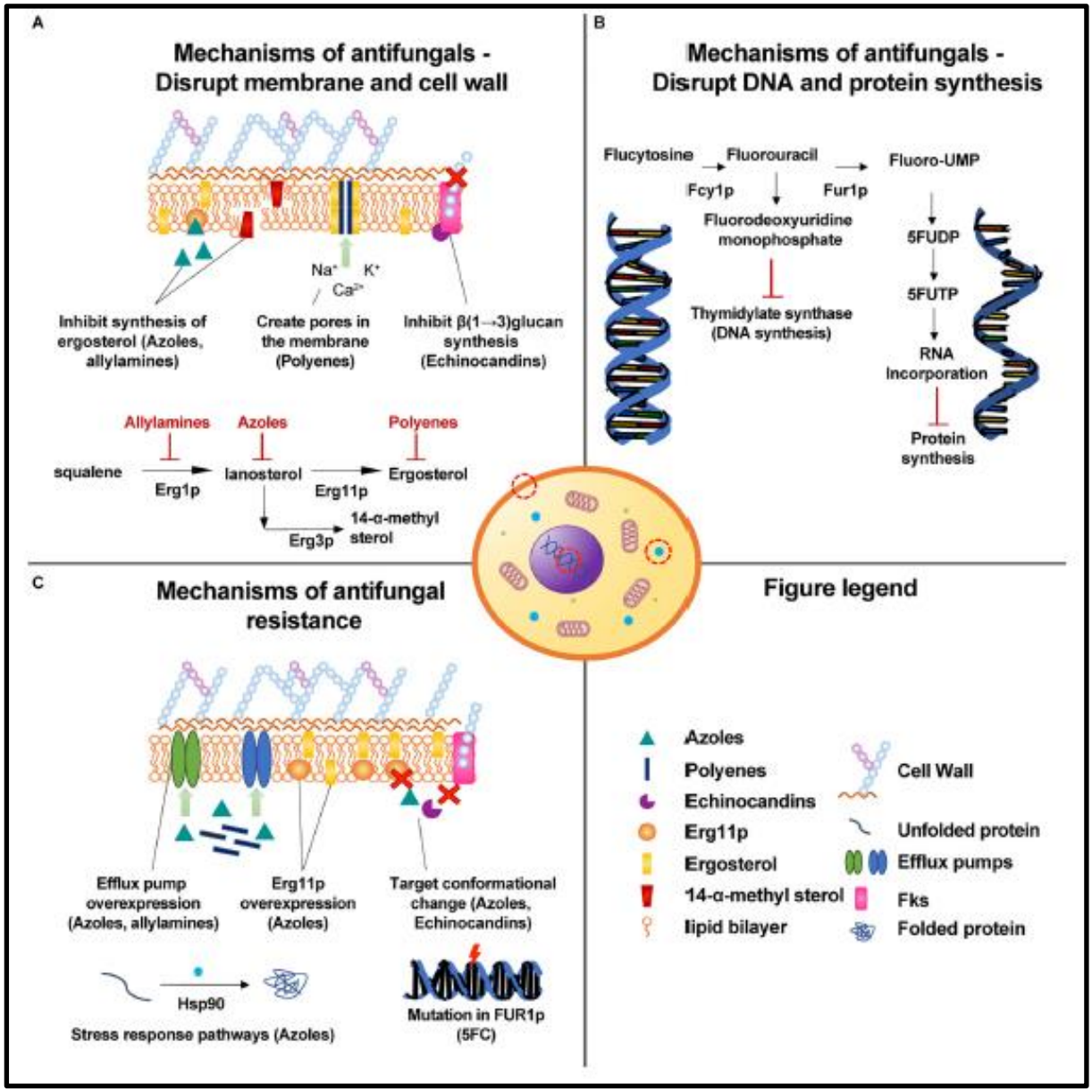

Figure 1. Mechanisms of drug action and resistance observed in *C. auris*. (A) The main mechanisms of antifungals disrupt the cell membrane or cell wall. (B) In the nucleus, 5-flucytosine inhibits the synthesis of fungal DNA and RNA. (C) Mechanisms of antifungal resistance to drugs that damage the cell membrane or cell wall (extracted from [11], with permission of Frontiers under the terms of the Creative Commons Attribution License).

Table 1. Drug resistance mechanisms in *C. auris.*

Effect	References	Comments
Drug target mutation	[13]	Mutations in some genes (*ERG11* and *FKS1*) have accounted for fluconazole action in *C. albicans* and, most recently, in *C. auris*. Antibiotic therapies have been facing many difficulties due to multidrug resistance. New antifungal drugs are recommended to control and prevent *C. auris* infections.
Drug target overexpression	[14]	Replicative aging (RA) can lead to fluconazole tolerance in *C. auris* cells due to transient gene duplication in old yeasts. This phenomenon was observed in other species such as *C. glabatra*, which presented similar resistance mechanism. Information about complete genetic mapping is relevant to help better understanding fluconazole resistance emergence.
Activation of stress response pathways	[15]	Another mechanism associated with fungal tolerance and resistance to azole was proposed in this study involving the Hsp90 molecular chaperone and *CDR1* gene. Hsp90 regulates morphogenesis of fungal pathogens in azole-induced cell membrane stress by repressing filamentous growth and by leading to tolerance to azole. Resistance to fluconazole is mediated by the *CDR1* ABC transporter gene regardless of Hsp90.
Biofilm formation	[16, 17]	Biofilm is an important factor hindering antimicrobial activity. Transcriptomic analysis has shown that some genes (adhesins and efflux pumps) were associated with biofilm-mediated resistance.
Changes in drug uptake and efflux	[18, 19]	Clades I, II, III, IV, and V are genetic groups of *C. auris* observed in the investigated geographic regions. Clades I, III, and IV are associated with multidrug-resistant infections caused by *C. auris*. Subtelomeric deletions in chromosomes associated with cell wall proteins and adhesins have suggested variation in some phenotypical properties (biofilm, adhesion, invasion, and damage) in Clade-specific adhesins. Transporter, efflux pumps, transcription factor, cell wall, and drug target genes have shown clade-specific selection signatures.

Despite the great challenge of finding new antifungal drugs against *C. auris*, a small number of new antifungal drugs have shown successful outcomes in clinical trials and may be accessible to be used in hospital settings in the next years [11, 20] (**Table 2**).

Table 2. New antifungals in trials.

Drug	Company	Trial (Phase)	Activity	Reference
Fosmanogepix (APX001)	Amplyx	*C. auris* (Ib), Candidemia (II)	Gwt1 inhibitor (novel)	[21] Hager *et al*., 2018 NCT04148287
Brexafungerp	Synexis	*C. auris* (III)	Glucan synthase inhibitor (novel, orally available)	[22,23] Berkow and Lockhart, 2017; Larkin *et al*., 2019
VT-1598	NQP 1598	*C. auris* (I)	CYP51 (Erg11p) inhibitor	[24] Wiederhold *et al*., 2019
VT-1161	Mycovia	Candidiasis (III)	CYP51 (Erg11p) inhibitor	[25] Brand *et al.*, 2018, p. 2
Rezafungin	Cidara	Candidemia (III)	Long half-life echinocandin	[26]Lepak *et al*., 2018
SCY-078	Scynexis	*C. auris*. Candidiasis, Invasive (III)	Efficacy, safety, tolerability, and PK (pharmacokinetics) of oral SCY-078	[27] Angulo D. NCT03363841
ATI-2307 (Arylamidine)	Appili	Phase I trials targeting future trials for cryptococcosis and MDR *C. auris*	MDR invasive candidiasis (IC)	[28] Nishikawa *et al*., 2017
PC945 (Triazole)	Pulmocide	Triazole-resistant *C. auris* (II)	Inhibitor of CYP5	[29] Murray *et al*., 2020

The strategy used by *Candida* spp. to increase its resistance lies on aggregating itself into a colony to produce biofilm [30]. Molecular chaperones belonging to family Hsp90 are essential regulators of biofilm dispersion, survival of fungi subjected to antibiotic factors (tolerance), and cell wall improvement [31]. For example, *C. auris* Hsp90 was capable of stimulating cell wall integrity signaling and stress responses associated with azoles' application and enabled drug resistance evolution [15].

Since *C. auris* is a resistant pathogen, it survives to rough decontaminations, desiccation, and salty moiety. Its efficiency to colonize vulnerable patients and to cause invasive contamination is one of the worst aspects of this microorganism. *C. auris* can be wrongly identified in traditional microbiology assays and this could generate a fast multiple genetic determinants that give to multidrug resistance. Actually, total-genome sequencing has featured four different *C. auris* clades in circulation in the world. However, since this pathogen grows in different clades worldwide, its emergence remains unexplained. According to [32], host-pathogen–environmental factors have evolved along adverse or unfavorable trajectories since the 2000s, mainly in countries *C. auris* originally emerged from, until all these factors possibly achieved a deflection point to force *C. auris* evolution, appearance and outspread. Although extended comparative genomics enabled identifying the several resistance mechanisms in *C. auris*, it failed to fully explain the fast emergence of high-level resistance in this microorganism.

Increased pathogen resistance identified in bacteria, fungi and parasites worldwide has led to shortage of antimicrobial drugs used to treat several pathologies in the last decades [33]. In addition, the current drug discovery pipeline has shown two-third of poorly soluble molecules under physiological conditions and consequent administration issues (*i.e.*, molecular aggregation, unpredictable kinetics, incomplete absorption, among others) [34]. Moreover, research processes adopted to place a single drug in the market are expensive and time-consuming, since it takes at least 10 to 15 years and approximately 10,000 molecule candidates to get to the ideal one, but only one or two molecules are approved by regulatory agencies. Drug repositioning is an alternative used to treat drug-resistant microorganisms since less than 400 genes are currently used as drug targets [35]. Based on a recent review, the combination of 124 antifungal drugs to different biocide molecules against *C. auris* was tested *in-vitro* [36]. Data compilation *in-vitro* has shown that only 36% of combined antifungal drugs were effective against *C. auris*, but approximately 16% of them were antagonistic. This approach is also limited by the progression of different *C. auris* pathologies since microbial overexposure to drugs leads to development of resistance mechanisms.

Another issue lies on drug solubility under physiological conditions since most drugs available in the market are insoluble. Several new approaches were recently developed to increase the bioavailability of poorly water-soluble drugs such as supercritical fluids, micronization, ionic liquid, among others [37, 38, 39]. However, most of these techniques are complex and/or require complex physicochemical approaches.

On the other hand, the emergence of nanotechnologies brings a myriad of new alternatives and strategies to overcome drug solubility, administration and toxicity issues.

The aims of the present study were to present new advances in *C. auris* treatment based on the currently available therapies, as well as to introduce the prospect of new nanotechnology advances.

Nanotechnology strategies

Nanostructures are promising approaches used to eradicate drug-resistant and invasive fungal pathologies since they can bind to and penetrate cells to effectively release the drug they were loaded with. Several studies have reported the use of metallic, polymeric, and lipid nanodevices to treat *Candida* infections [1, 40, 41, 42]. Initially, the use of different antifungal nanoparticles was mostly tested in *C. albicans*, which is the main infective species producing candidiasis. For example, the semisolid lipid, Gelucire®, was used to produce microparticles loaded with chitosan or carboxymethyl cellulose and/or poloxamers loaded with econazole nitrate to treat vaginal infections produced by *C. albicans*. Results have indicated microparticles' high mucoadhesive properties and effective yeast growth inhibition under low administration frequency [43]. Based on another study, silver nanoparticles (mean size = 5 nm) synthesized in the presence of citrate and capped with ammonia were tested on biofilms; they adhered to *C. albicans* and *C. glabrata* cells and showed MIC ranging from 0.4 to 3.3 μg mL^{-1}. However, AgNPs were only highly effective at early cell adhesion stages and before biofilm formation [44]. Later, chitosan nanoparticles loaded with miconazole nitrate (mean size = 207.3 ± 0.8 nm) were successfully challenged against vaginal infections caused by *C. albicans*. In addition, they enabled reducing by seven times the antifungal drug concentration [41]. These seminal studies were some of the platforms used to challenge drug-resistant *C. auris* based on new strategies and more sophisticated nanodevices.

Microwave-assisted AgNP synthesis against *C. auris* has indicated high inhibitory activity at IC_{50} of 0.06 μg mL^{-1} (biofilm formation) and 0.48 μg/mL (IC_{50} of preformed biofilms). Stained biofilm pictures taken through Scanning Electron Microscopy (SEM) have shown cell wall damage, mainly due to breakage and distortion in the outer surface of the fungal cell wall. Silicone elastomer decorated with AgNPs has shown biofilm suppression (>50%, 2.3 up to 0.28 μg mL^{-1}). Wound dressings impregnated with AgNPs have suppressed *C. auris* biofilm growth (> 80%, 2.3 up to 0.017 μg mL^{-1}). AgNPs-decorated fibers have kept their fungicidal effect, even after several washes [45].

The antimicrobial activity of chemically synthesized AgNPs in *C. auris* planktonic and biofilm growth stages in four different clades has shown MIC values for AgNPs used against different fungal strains of approximately < 0.5 μg mL^{-1} (under planktonic conditions), whereas values recorded for biofilm formation suppression reached approximately < 2 μg mL^{-1} for all tested strains. It also acted in preformed biofilms deriving from all tested *C. auris* strains and presented IC_{50} values ranging from 1.2 μg ml^{-1} to 6.2 μg ml^{-1} [46].

Gangadoo *et al.* [47] reported the production of long-term microbicidal silver nanoparticle clusters (AgNP@Cu). The AgNP@Cu coating was generated on copper surface by using ion exchange and reductant in order to obtain a reductive reaction. The nanostructured material surface was infected with *C. auris*, whose growth was monitored at different periods-of-time. It was found that after a week >90% of *C. auris* evidenced to be non-viable on the newly designed surface.

Results similar to those recorded for AgNPs used against *C. auris* [46] were observed for chemically synthesized bismuth nanoparticles (BiNPs) used against multidrug-resistant yeast species *C. auris*, both under planktonic and biofilm growth requirements [48]. BiNPs MIC values under planktonic conditions ranged from 1 μg mL^{-1} to 4 μg mL^{-1} in all strains tested with different clades. BiNPs IC_{50} values recorded for biofilm formation ranged from 5.1 μg mL^{-1} to 113.1 μg mL^{-1}. Moderate activity against biofilm growth was observed in the latter case.

Biogenic silver nanoparticles (AgNPs) and their functionalization in chitosan matrix (AgNPs@Chi) against *Candida* spp. were recently investigated [49]. Several *Candida* species have shown significant minimum inhibitory concentrations (MIC ranged from 0.06 μg mL^{-1} to 1.00 μg mL^{-1}). It is known that nanocomposite-treated cells have shown cytoplasmic

degeneration and membrane/wall cells breakage. AgNPs@Chi had additive effect on *Candida* spp. in the presence of fluconazole and amphotericin B. Nanotoxicological studies have evidenced low toxic effect on mammalian cells and on *Galleria mellonella* larvae, and it suggested the likelihood of using them *in vivo* against *Candida* infection. Murine cutaneous candidiasis treated with AgNPs@Chi presented reduced skin fungal infection and fast tissue recovery due to dose-response action.

Recent study has tested PBS-loaded bacterial cellulose (BC) hydrogels, Hydroxypropyl-β-cyclodextrin (HPβCD)-loaded BC and curcumin- hydroxypropyl-β-cyclodextrin (CUR: HPβCD)@Ag (cAgNP)-loaded BC hydrogels against *C. auris* based on 24-h disc diffusion assay. PBS-loaded BC and HPβCD-loaded BC did not present antimicrobial activity, whereas cAgNP-loaded BC has shown effective antimicrobial activity ($p< 0.001$) against *C. auris* [50]. Sherin and Kuriakose [51] reported synthesis of superparamagnetic Fe_2O_3 nanoparticles stabilized by biocompatible supramolecular β-cyclodextrin; they were tested against *C. auris* and presented inhibition zone size of 36.67±0.82 mm, although with moderate MIC= 500 µg mL^{-1}.

Beta vulgaris extracts were used to synthesize Ag-Fe nanoparticles and showed antifungal activity against 25 different *C. auris* strains [52]. Ag-Fe-NP has shown mean size of 14.30±2.20 nm, spherical shape, MIC values ranging from 019 µg mL^{-1} to 039 µg mL^{-1}, and Minimal Fungicide Concentration (MFC) ranging from µg ml^{-1} 039 to 0.78 µg ml^{-1} against 25 clinical *C. auris* isolates. MIC and MFC values recorded for Ag-Fe-NP were lower than those of amphotericin MICs and MFCs in all cases. Ag-Fe-NPs' lethal effect on *C. auris* can be attributed to oxidative stress, since it caused cell cycle inhibition in the G2/M phase, which was followed by cell apoptosis [53]. Based on another study conducted by the same research group, biogenic Ag-Cu-Co trimetallic nanoparticles have shown minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) ranging from 0.39 µg mL^{-1} to 0.78 µg mL^{-1} and from 0.78 µg mL^{-1} to1.56 µg mL^{-1}, respectively. Mechanistic analysis has shown that these trimetallic nanoparticles could cause apoptosis and stop G2/M phase cell cycle in *C. auris*. Moreover, Ag-Cu-Co trimetallic nanoparticles presented increased antimicrobial properties in comparison to their monometallic counterparts (*e.g.*, Ag, Cu, and Co). Therefore, the aforementioned study has shown that Ag-Cu-Co trimetallic nanoparticles had potential to be used as antifungal drug against *C. auris* contaminations [53].

Clearle *et al.* [54] conducted an experiment with six *C. auris* strains in the presence of nitric oxide donor, namely: N-acetylcysteine S-nitrosothiol nanoparticles (NAC-SNO-NP); which are capable of releasing N-acetylcysteine S-nitrosothiol (NAC-SNO) and N-acetylcysteine (NAC) to produce NO. Results have shown that NAC-SNO-NP has effectively eliminated C. *auris* under planktonic and biofilm conditions. Treatment with 10 mg mL^{-1} of NAC-SNO-NP applied to all six strains has effectively reduced the number of colony-forming units (CFUs) and showed decrease by >70% in biofilm feasibility. Thus, NAC-SNO-NP has effectively eliminated planktonic *C. auris* and significantly minimized *C. auris* biofilm formation.

Biopolymer using is an easy strategy adopted for drug nanoencapsulation purposes. Alginate is a GRAS biopolymer produced by brown seaweed algae externally emulsified with sunflower oil and SPAN 80. Miltefosine (hexadecylphosphocholine, MFS) nanoentrapment in alginate nanoparticle gels (AN) was tested *in vitro* and *in vivo* by using *Galleria mellonella* larvae against 45 clinical *C. auris* isolates [55]. Although MFS is a broad-spectrum antibiotic with fungicidal activity, it presents several toxic effects such as hepatotoxicity, hemolytic activity, and gastrointestinal irritation, which limits its use in patients for long periods-of-time. MFS nanoencapsulation appears to be an alternative to avoid its secondary effect, increase its effectiveness, and extend its half-life. MFS encapsulation reached approximately 80%

efficiency in AN showing mean size 279±56.7 nm, low polydispersity (0.42±0.15), and high stability (39.7±5.2 mV). The ANP-MFS has shown controlled release kinetic of approximately 4.68% and 7.55%, after 6 h and 24 h, respectively. MFS was hemolytic (50%) at concentrations close to 35 μg mL^{-1}, 128 μg ml^{-1} MFS and did not show sign of hemolysis in AN [56]. MFS presented suppressed effect *in vitro*, MICs of approximately 1–4 μgmL^{-1}, and fungicidal action (2 μg mL^{-1}) against planktonic cells of clinical *C. auris* isolates. MFS antibiofilm action was observed in biofilm formation (from 0.25 μg mL^{-1} to 4 μg mL^{-1}) and in preformed biofilms (from 16 μg mL^{-1} to 32 μg mL^{-1}). Furthermore, dissipated *C. auris* biofilm cells presented sensibility similar to that observed for planktonic cells. On the other hand, MFS-AN recorded MIC value of lower inhibitory by 600-times for *C. albicans, C. krusei, C. tropicalis and C. parapsilosis* strains, but not for any *C. auris* strain [55, 56]. Assays conducted *in vivo* at MFS doses higher than 50 mg kg^{-1} have shown 77.8% *G. mellonella* larvae survival, whereas AN doses of 100 mg kg^{-1} and 200 mg kg^{-1} did not increase larval mortality rates. Treatment with free MFS or MFS alginate nanoparticles (MFS-AN) has effectively increased the survival and morbidity rate of *Galleria mellonella* larvae contaminated with *C. auris*. Besides these results, reduced fungal loading (0.5–1 log CFU g^{-1}) and granuloma generation related to the untreated group were observed. Barretos *et at*. [55] suggested that either free MFS or MFS-AN has the potential to be used as drugs to treat fungal infections induced by *C. auris* emergence. These results have indicated that the use of MFS entrapment in alginate nanoparticles led to extended drug release without toxicity symptoms.

Hamdy *et al.* [57] have investigated the chemical combination of cuminaldehyde oil and azole intermediates (such as the ones found in sulconazole or voriconazole) (UoST) in order to use their components, which had been previously reported as effective anticandidal drugs, in a UoST series against *C. auris*.

UoST5 (Fig.2) was the best among all tested UoST series – it recorded MIC value of 2 μg mL^{-1}. Compound UoST5 was also formulated in polymeric nanoparticles of poly (lactic-co-glycolic acid) (PLGA); this formulation was capable of enhancing and extending UoST5's anticandidal activity (**Figure 2**).

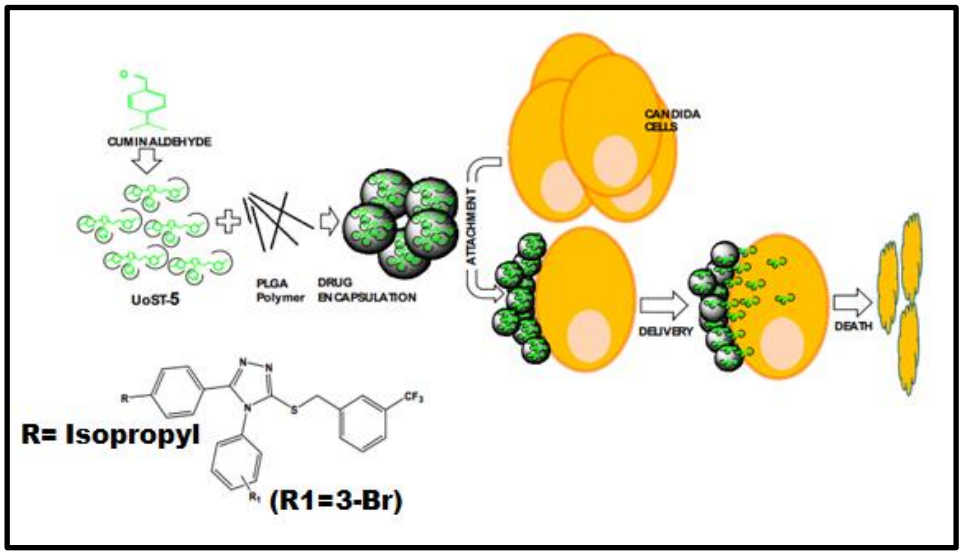

Figure 2. Graphical representation summarizes the synthesis, formulation, and proposed mechanism of attachment and delivery of UoST5-NPs (extracted and modified from [57]; under Creative Commons Attribution License. From MDPI Press)

A new multi-ethosome (ME) system for the dermal delivery of terbinafine hydrochloride (TH) as novel approach to fungal infection therapy was reported by [58]. Ethosome resulted from changes in liposomes added with ethanol. The classical ethosome (CE) is formed through ethanol addition to the inner aqueous phase and to the lipid bilayer, and it leads to liposome deformability. In addition, ethanol decreases water activity, which is relevant to reduce fungal growth and, consequently, to increase TH activity. ME's enhanced antifungal action was evidenced in *Candida albicans* strains based on MIC assay conducted *in vitro* (0.0156–0.125 $\mu g\ mL^{-1}$). The aforementioned research opened room to the use of MEs for dermal fungal contamination treatment with high likelihood to obtain successful action on *C. auris*.

Natural bioactive compounds such as carvacrol, cinnamaldehyde, citral, thymol, and their derivatives, were reported to have antifungal activity against *Candida* spp. [59, 60, 61]. The antifungal mechanisms described for these bioactive compounds comprised ATPases' inhibition, disruption of the ergosterol biosynthetic pathway involved in membrane structure, and cell wall biosynthesis interference. All these compounds are oily and, consequently, they present low solubility in aqueous environments, which hinders their administration and biological activity. A research group has developed dioctadecyl dimethyl ammonium bromide and monoolein liposomes for carvacrol, cinnamaldehyde, citral, thymol dissolved in DMSO, and tested their antifungal activity in four clinical *Candida* spp. isolates, including *C. auris.* The authors of the aforementioned research have suggested that liposomal formulations presenting natural oily molecules showed low toxicity and activated macrophages to kill *Candida* spp. specimens. The best results were recorded for liposomal formulations comprising carvacrol and thymol incubated with macrophages; they reduced fungal survival rate by approximately 41% [62]. The limited antifungal activity of the liposomal formulation could be likely improved by changing the platform into solid lipid nanoparticles, since liposomal formulations are thermodynamically unstable. In addition, their composition, most of their physicochemical parameters and kinetic drug release depend on the environmental conditions.

Farnesol is an essential oil extracted from flowers of different origins; it is chemically defined as acyclic sesquiterpene alcohol with biological properties such as antibacterial and acaricide activity. In addition, farnesol was reported to be capable of activating T-cells by binding to CD1a molecules exposed in Langerhans cells. Previous research has shown that farnesol is produced by *Candida* spp. and linked to the quorum sensing mechanism adopted to prevent filamentation, but it also plays relevant role in cellular competence, biofilm production decrease and reduce *Candida* spp. virulence reduction [63, 64]. Most recently, research groups have investigated the role played by farnesol in *C. auris* metabolism and physiology, as well as its potential synergism with antifungal drugs [65, 66, 67]. Interestingly, 125 mM farnesol has inhibited fungal adhesion to different surfaces and biofilm formation in 16 of 25 clinical *C. auris* isolates in South Africa, a fact that made cells more susceptible to antifungals [57]. Based on another study, the biological activity of farnesol was concentration-dependent in the range from 100 mM to 300 mM, a fact that led to planktonic *C. auris* cell inhibition and synergically enhanced the antifungal activity of echinocandins, which is one of the preferred antifungal drugs available in the market [65, 66]. In that very same year, farnesol was formulated in liposomes to test its antifungal activity against *C. albicans*, *C. tropicalis,* and *C. krusei* in the presence of fluconazole. Results have indicated that farnesol is antagonistic to fluconazole, but the antifungal activity of liposomal farnesol combined to fluconazole was approximately 22 and 470 times more effective in *C. albicans* and *C. tropicalis*, respectively. However, it is not recommended for *C. kruzei* because its biocide activity decreased by approximately 2 times [68]. Since, according to the CDC (Central of Control Diseases, USA), fluconazole is not effective against most *C. auris* strains, the effect of encapsulated farnesol should be analyzed in the presence of other antifungals. However, the antifungal activity of

encapsulated farnesol appears to be an attractive strategy to be adopted in drug-resistant yeast therapies.

Amphotericin B (AmB) is a natural polyene and one of the antifungal drugs mostly used to treat candidiasis. However, AmB belongs to BCS class IV (biopharmaceutics classification system) since its low solubility and low permeability lead to administration issues. In addition, Amb presents undesirable secondary effects such as chronic nephrotoxicity and hemotoxicity, vomit, fever, hypoxia which are limited extensively clinical uses [69]. Some commercial formulations capable of reducing AmB toxicity and administration issues are currently available in the market, namely: Abelcet®, which is an AmB lipid complex; Ambisome®, which is a liposomal AmB; Amphotec®, which is a colloidal dispersion of AmB; and Fungizome®, which is an AmB deoxycholate [70]. Besides, all traded formulations presented some concern such as low drug loading, fast release, and limited effectiveness in studies conducted *in vivo* [71]. In addition, the effectiveness of commercial formulations depends on the pathology, infective strain and patients' health condition. However, the commercial liposomal formulation of AmB (*i.e.*, Ambisome) was reported to be more effective than the free AmB as antifungal [72].

Rodriguez *et al.* [73] have investigated micellar systems used for AmB application and used amphiphilic block copolymers (ABCs) combined to retinol. AmB encapsulated in polymeric micelles has shown enhanced antifungal effectiveness; it recorded MIC values ranging from 0.93 to 1.865 mg L^{-1} against *C. auris*, in comparison to the MIC value (3.75 mg L^{-1}) recorded for Fungizone®. Therefore, it is likely that infections induced by amphotericin-B-resistant *C. auris* can be treated with the same antifungal drug, although it is more efficiently administered when it is encapsulated in polymeric micelles.

Traded AmB formulations presented significant differences in kinetic release and biocide activity against biofilms of different *C. auris* strains. However, all isolates (five strains were investigated, each strain belonged to a given *C. auris* clade, often referred to as East Asian, South Asian, African, Iranian and South American clades), presented relatively low planktonic cell (PLK) MICs (0.06-4 μg mL^{-1}) for both AmB formulations (*e.g.*, free and liposomal formulations). Liposomal AmB has shown profile different from that of deoxycholate AmB against *C. auris* biofilms since it recorded much higher biofilm (BF) MICs than that recorded for planktonic growth (16-512 μg mL^{-1}). Among nine known antifungal agents (fluconazole, posaconazole, itraconazole, voriconazole, caspofungin, anidulafungin, micafungin, deoxycholate, and liposomal AmB), deoxycholate AmB (2-4 mg L^{-1}) was the only drug presenting BF MICs close to the corresponding PLK MICs [74]. Echinocandin and liposomal AmB are often suggested as first-line drugs for most patients with *C. auris* isolated in urine samples, based on infection control analyses and on adequate follow-up guidelines [75].

Since *Candida* spp. can form resilient films on different surfaces that require high drug concentrations and/or drug cocktails, the new strategy lied on immobilizing liposomal AmB on surface composed of polydimethylsiloxane polymerized with dopamine. The resulting surface was more hydrophilic and lesser rough; it prevented *Candida* cell adhesion to it and was capable of killing fungal cells in contact with the AmB liposome [76]. Because liposomes are thermodynamically unstable, liposome immobilization has also the advantage of keeping its mean size and structure, which allows predicting AmB release from liposome surface. This nanotechnology is relevant since it can be applied in medical equipment such as catheters, protective equipment, mainly in ventilator connectors used for oxygen administration in SARS-CoV-2 patients subjected to corticoid therapy. Another recent strategy lied on developing nanostructured film made of boron nitride (BN) nanoparticles composed of nanosheets and nanoneedles (15 nm, in thickness) and loaded with gentamicin and AmB. The antibiotic-loaded BN films can kill both bacteria and fungi. In addition, BN films immersed in saline aqueous

solutions release reactive oxygen species that can damage cell membranes due to oxidative stress, which can be synergic with the antibiotics [77].

A relevant strategy used to reduce AmB toxicity lied on combination with AgNPs, which is a well-known biocide [40, 78]. Biogenic AgNPs chemically capped with AmB were tested on agar plates against *Candida albicans* and *C. tropicalis* and showed high growth inhibition haloes of 16 ± 1.4 mm and 18 ± 1.5 mm, respectively [40]. According to another research, AmB capped with AgNPs has shown mean nanoparticle diameter of 11 nm on several fungi. MIC and MFC values recorded for AmB-AgNPs against *C. albicans* were 0.25 µg mL^{-1} and 2 µg mL^{-1}, respectively. On the other hand, commercial AmB (*i.e.*, Fungizone®) used at the same concentrations did not show inhibitory effect on fungi. Simultaneous addition of AmB and AgNPs has shown high MIC and MFC against *C. albicans* and other fungi in comparison to values recorded for AmB-coated silver nanoparticles (AmB-AgNPs), which have shown strong binding to fungi hyphae surface, as shown in Raman map. Moreover, the toxicity of AmB-AgNPs tested on human colon epithelial cells (CCD-841CoTr) and human monocytes (THP-1) was lower than that of the commercial fungicide [78]. These studies conducted with *C. albicans in vitro* were conclusive about the superior inhibitory effect presented by AgNPs associated with AmB. Furthermore, recent review about the production of nanotechnology devices for AmB delivery in fungal and parasite pathologies has evidenced the advantages of using these novel approaches for therapeutic purposes [79].

Recent study conducted by Shtansky lab has shown that silver-boron nitrite hybrid nanoparticles (Ag-BN-nps) with mean size ranging from 3 nm to 15 nm, and loaded with 20% AmB have increased their size at range from 170 nm to 280 nm, based on antifungal drug concentration. The Ag-BN-AmB nanohybrid has shown strong antifungal activity against several *Candida* spp. strains, including *C. auris*. Interestingly, the authors of the aforementioned study have suggested that AmB was firstly adsorbed on Ag and, later, this interaction was displaced to BN-nps. The postulated mechanism of AmB binding to Ag nanohybrid particles is relevant since AgNPs keep the biocide activity that could present synergic activity with the antifungal drug.

Dennis *et al*. [81] have synthesized and featured six gold(I)-phosphine (GP) complexes. GP's antifungal activity was tested in 22 *Candida* species (*i.e.*, *C. albicans* (7), *C. auris* (12), *C. glabrata* (1), *C. krusei* (1), *and C. parapsilosis* (1)). Two GP complexes have shown high antifungal activity in *C. auris*, which was comparable to that of AmB [81]. However, complex stability must be analyzed, since their toxicity is a significant limitation for their administration. The development of nanodevices designed to encapsulate GP complexes may not only reduce their toxicity but also enhance their antifungal activity against *Candida* spp.

Another advantage of using drug-loaded nanoparticles lies on the potential reduction and/or elimination of drug-resistant mechanisms. For example, lipid-based nanoparticles loaded with fluconazole (FCZ) are often used in antifungal therapies, although they show low effectiveness in many cases due to drug-resistance mechanisms observed in several *Candida* spp. strains. Two lipid formulations added with fluconazole were developed: one of them was a polymeric nanoparticle presenting lipid core coated with caprolactone (LCN) and the other one was a solid lipid nanoparticle (NLC) [82]. Nanoparticle diameters of LCN and NLC were 211 nm and 136 nm, and their pH was 4.88 and 6.70, respectively. NLC was not capable of reverting FCZ resistance to *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei*, whereas LCN has shown better antifungal activity than free FCZ. Results have shown that nanoparticles' diameter is not the only requirement to overcome drug-resistant mechanisms. Most specifically, nanoparticles' surface properties can contribute to enhanced binding to fungal cell wall. Polycaprolactone (the LCN coat) is a hydrophobic polymer capable of improving

nanoparticle binding of LCN to the fungal surface; consequently, it increases the number of nanoparticles attached to fungi and reduces the effective FCZ doses.

Natural compounds produced by plants, such as quercetin and curcumin, are excellent antimicrobials and antioxidants; they present wound-healing properties. Niosomes composed of cholesterol and non-ionic surfactants (*i.e.*, sorbitan derivatives, (1-Hexadecyl) trimethylammonium bromide) were statistically optimized for quercetin and curcumin encapsulation [83]. In another study, the same research group has challenged 10 Candida spp. strains, three of them were *C. auris* presenting niosomes loaded with quercetin and curcumin. Mean MIC values recorded for niosomes loaded with quercetin and curcumin and used against *C. albicans*, *C. auris*, and *C. glabrata* were 2.5, 5.0, and 7.6 μg mL^{-1}, respectively [84]. The authors of the aforementioned study have claimed that the niosome formulation added with quercetin and curcumin was approximately 3.4- and 8.0-times more efficient than fluconazole to treat fungal skin infections.

Nowadays, the pipeline presents several molecular candidates capable of producing antifungal effects in synergy with commercial antibiotics or of presenting strong antifungal activity. Among them, one finds ceragenins [85], aprepitant [86], and 6-shogaol [87]. However, as previously stated, most new molecular candidates are hydrophobic compounds that represent significant issue due to potential aggregation in aqueous media, uncertain adsorption kinetic, low bioavailability, and toxic side effects, among other undesirable properties. Nanodevices will provide effective kinetic delivery of novel molecular candidates and allow combating drug-resistant pathogens in a more efficient way.

Final Remarks

Several advances in the development of nanodevices with therapeutic applications were reported in the last decades. Many of them can help avoiding drug solubility issues under physiological conditions, reducing drug toxicity due to targeting and/or established kinetic release, synergic activity between drug and matrix components, or by capping the nanodevices. Most specifically, nanoparticles are a very attractive alternative against drug-resistant microorganisms such as *C. auris*, mainly after the emergence of SARS-CoV-2. Nanodevices combined to antifungal drugs can show synergic biocide effect, as well as lower the drug concentration required to eliminate pathogens. New strategies focused on developing nanodevices must encompass more sophisticated molecular structures, such as chaotropic and surface-active compounds, enzymes, bioactive polymers, natural products, as well as other bioactive molecules capable of increasing the effectiveness of treatments and mitigating undesirable side effects.

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