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Synergistic Eeffect of Polyene Antifungals and Silver Nanoparticles Against *Candida Parapsilosis*

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Abstract

Candidiasis is a fungal infection caused by yeasts of the genus *Candida* spp. Among several pathogenic *Candida* species, we can highlight the *C. parapsilosis* due to susceptibility profile. Amphotericin B and nystatin are polyene antifungal drugs most frequently used to combat this type of infection. Silver nanoparticles (AgNPs) also have antifungal properties and can also provide synergistic action when combined with classic antifungals. Therefore, the aim of this study was evaluate the synergistic action of AgNPs, amphotericin B and nystatin against *C. parapsilosis*. The synthesis of AgNPs were performed with glucose, and sodium dodecyl sulfate (SDS) was used as a stabilizer. The characterization of AgNPs was performed by scanning electron microscopy (SEM) and atomic force microscopy (AFM). It was found a value of 18.17 nm (±5.3) for the size of the AgNPs, when measured by AFM. AgNPs were shown to be quite uniform and stable, and when combined with amphotericin B and nystatin, showed potent antifungal activity and increased the zone around the antifungal disk by 222.6 and 319.3%, respectively. These results contribute to a better understanding of the synergistic effect and offers another option for the treatment of fungal infections.

Keywords: *C. parapsilosis;* Silver Nanoparticles; Polyene Antifungals; Synergistic Effect

Introduction

Infections caused by yeasts of the genus *Candida* are highly relevant due to their high morbidity and mortality rates, which can be compared to septic shock [1,2]. Infections caused by *Candida* spp. include about 80% of all fungal infections reported in hospitals; blood, urine and surgical wounds are the main body sites involved. *Candida parapsilosis* is an yeast constantly isolated from candidemia and exhibits mortality rates that are considered high. Its identification is no simple task, requiring biochemical, phenotypic and molecular methods [1].

There are many types of drugs used to treat fungal infections, such as the polyene antibiotics, which are broad-spectrum antifungals such as nystatin and amphotericin B. The former is part of many topical preparations available on the market, and the latter is an intravenous drug, widely used in combating candidemia [3]. The continuous use of these antifungals may promote the selection of resistant pathogenic fungi, not controlled by the previously effective fungicide, thereby jeopardizing its efficacy. Therefore, several strategies can be used to prevent or reduce fungal resistance, and one of the most common ones recently is to evaluate the synergism of antifungal drugs with other substances: essential oils, formulations with other antibiotics, and drugs without antifungal indication [4-7].

Silver nanoparticles (AgNPs) are structures with sizes up to 100 nm and exhibit different properties than those found in the source material. AgNPs are unstable and their synthesis involves many products, sometimes toxic, as the preparation methods can be chemical, biological or physical [8,9]. This kind of material has emerged as a promising and potent treatment against microorganisms due to its antibacterial, antiviral and antifungal effects, which are described in several studies [10,11]. In this way,

the purpose of this study was evaluate the synergistic action between two types of polyene antibiotics (nystatin and amphotericin B) associated with AgNPs against strains of *C. parapsilosis* isolated from candidemia.

Materials and Methods

Origin of the strains

Ten strains of *C. parapsilosis* isolated from candidemia of patients admitted to public hospitals in Fortaleza, Ceará, in Northeastern Brazil, were used in this study. These were identified by biochemical, phenotypic and molecular methods [12]. These yeasts belong to the collection of the Laboratory of Microbiology of the Faculty of Pharmacy of the Federal University of Ceará.

Production of AgNPs

The AgNPs were synthesized using a solution of 5 mM silver nitrate (Dinâmica - São Paulo - Brazil), 1.0g glucose (Dinâmica - São Paulo - Brazil) as a reducing agent, and 0.5g sodium dodecyl sulfate (SDS) (Vetec - Rio de Janeiro - Brazil) as a stabilizer [13].

Characterization of the AgNPs

The morphology, distribution and size of the AgNPs were determined using Scanning Electron Microscopy (SEM) and atomic force microscopy (AFM).

Evaluation of the synergism of amphotericin B and nystatin with AgNPs

The synergism effect was evaluated by the disk diffusion method on Mueller-Hinton medium (Himedia- Mumbai -India) supplemented with 2% glucose and 0.5 µg/mL methylene blue. We used commercial discs containing amphotericin B 100µg (AB)-(Cecon®) (São Paulo- Brazil), and nystatin 100 IU (NY)- (Cecon®) (São Paulo- Brazil). Each disc was impregnated with 10 µL of AgNPs. The disks were labeled as Ag@AB (disk containing amphotericin B and AgNP); Ag@NY (disk containing nystatin and AgNP); AB (disk containing only amphotericin B) and NY (disk containing only nystatin). Four discs were placed in each plate with *C. parapsilosis*. The plates were incubated at 35 ˚C for 24-48h and strains were tested three times [14].

In this study, synergism was considered as an increase in the area around the antifungal discs impregnated with AgNPs. The following formula was used: $(B^2-A^2)/A^2$ and $(D^2-C^2)/C^2$, where A, B, C and D are the inhibition zones of the disc of amphotericin B (A), Ag@ANF (B), nystatin (C) and Ag@ NYS (D). Increases in area were considered as synergism; no change was seen as indifferent, and decrease was regarded as antagonism [14,15].

Results

Figure 1 shows the characterization of AgNPs used in this study. As can be seen in the SEM (Figure 1 (a)) and AFM (Figure 1 (c)) images, the nanoparticles are well distributed with no agglomerations. The average size found by SEM (Figure 1(b)) and AFM (Figure 1(d)) were 24.04 nm (± 17.93) and 18.17 nm (± 5.83), on average (± standard deviation), respectively.

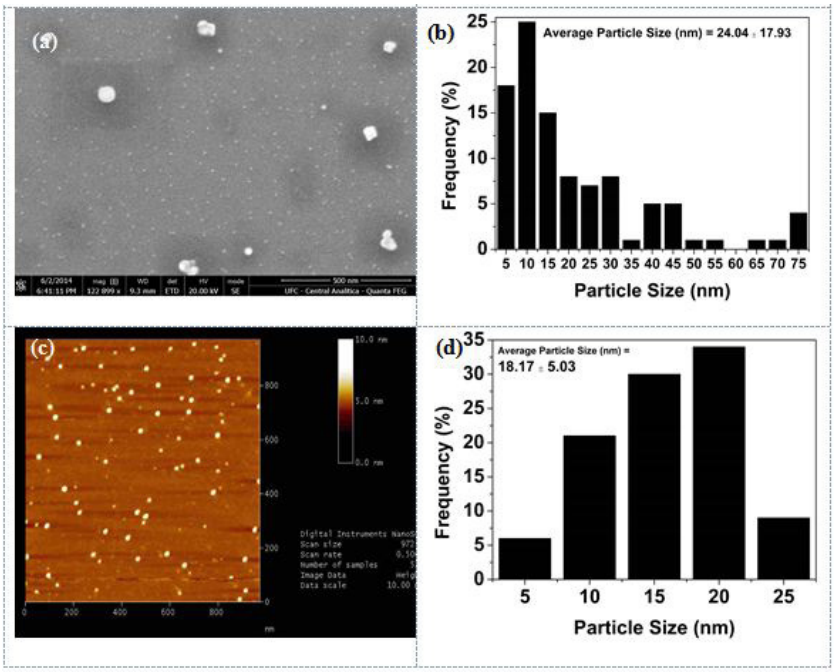

Figure 1: SEM (a), histogram obtained from SEM images (b), AFM (c) and histogram obtained from AFM images (d) of AgNPs

The identification of *C. parapsilosis* was carried out in a chromogenic medium. In such medium, this yeast exhibits a characteristic pink color, as shown in Figure 2 (a). The identification was confirmed in micromorphology. Figure 2 (b) shows high synergistic activity with the combination of amphotericin B and nystatin with AgNPs.

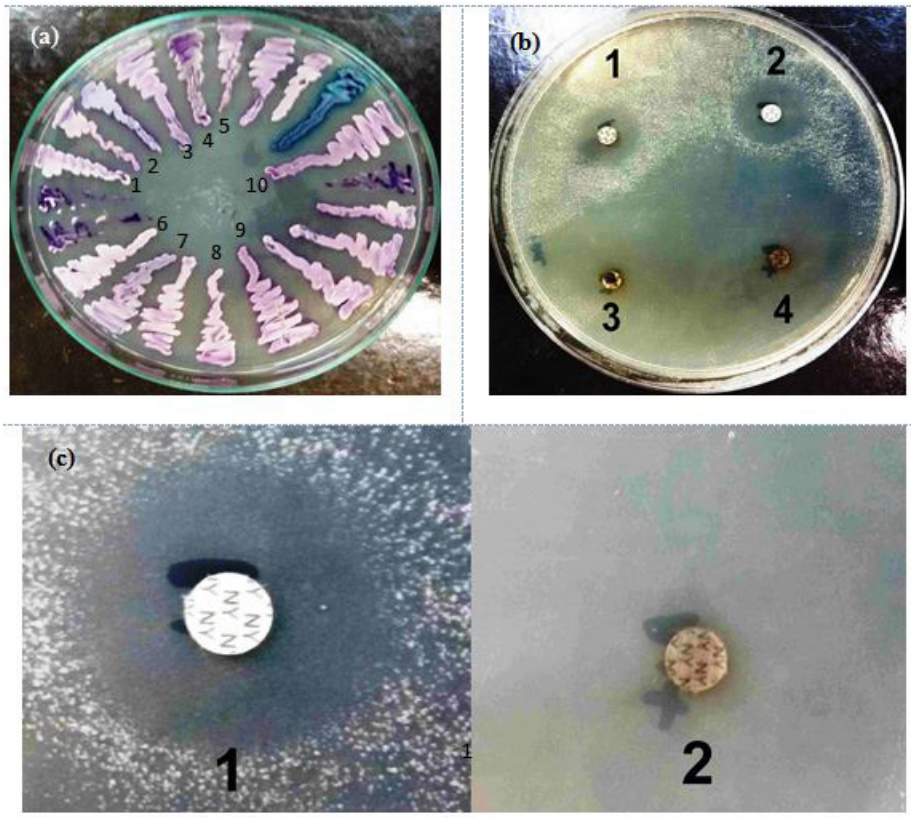

Figure 2: (a) *C. parapsilosis* on chromogenic medium (1-10). (b) Effect of polyene antifungals alone and associated with AgNPs: 1- amphotericin B alone; 2-nystatin alone; 3-Ag@AB; 4-Ag@NY. (c) Detail shows the effect of nystatin alone (1) and Ag@NY (2)

The disks impregnated with AgNPs showed an increased inhibition zone, which characterizes synergistic action. In Figure 2 (c), we can observe in detail the enlargement of the area around the nystatin disk (NY). This result can be observed numerically in Table 1 and, as can be seen in Figure 3, the combination of polyene antifungals and AgNPs were effective against *C. parapsilosis* and statistically significant (Student t-test). The enlargement of the area around the disks of antifungals impregnated with AgNPs was 222.6% for Ag@AB and 319.3% for Ag@ NY, when compared with discs containing only antifungal agents (Table 1).

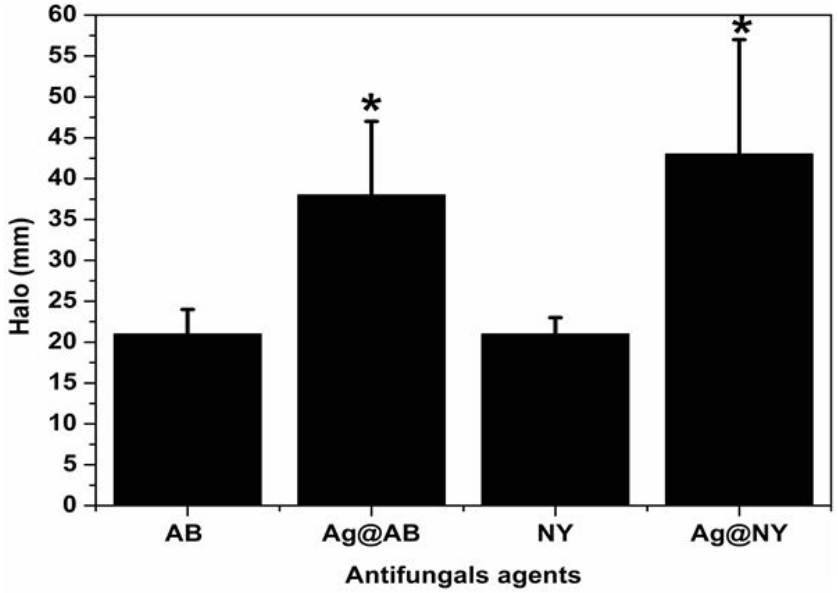

Figure 3: Effect of polyene antifungals alone and associated with AgNPs, AB- amphotericin B alone; NY-nystatin alone; 3-Ag@AB-Amphotericin B + AgNP; Ag@NY- Nystatin + AgNP. *Significantly different from antifungal discs $p<0.001$

Yeasts(n)	Halo (mm)				Increased fold area (%)	
	A	B	C	D	$(B^2 - A^2)/A^2$	$(D^2 - C^2)/C^2$
	AB	Ag@AB	NY	Ag@NY	Ag@AB	Ag@NY
C. parapsilosis (10)	21.1	37.9	21.0	43.0	222.6	319.3

AB- amphotericin B; Ag@AB- amphotericin B +AgNP; NY- Nystatin, Ag@NY- Nystatin+AgNP. Mean surface area of the inhibition zone (mm^2) was calculated for each combination tested from the mean diameter. Increased fold area was calculated using $(B^2 - A^2)/A^2$ and $(D^2 - C^2)/C^2$ (x 100), where A and B and C are the inhibition zones for A, B, C and D, respectively.

Table 1: Antifungal activity of AB, NY discs alone and Ag@AB, Ag@NY against *C. parapsilosis*

Discussion

Silver nanoparticles (AgNPs) can become a valuable ally in fighting resistant fungal infections [16]. The AgNPs produced in this study were shown to be stable and were obtained by a simple and inexpensive method (Figure 1).

In a study analyzing the synergistic action of AgNPs and antimicrobials against a group of bacteria, using the same method used in this study, the increase in area was evident, which demonstrates the potential of these compounds [14]. Studying the antifungal activity of AgNP and fluconazole, observed a powerful synergistic effect, primarily against *C. albicans*, analyzing the effect of the combination of AgNPs and nystatin against biofilms of *C. albicans* and *C. glabrata*, observed a synergistic effect and highlighted the importance of this combination [15,17]. Our results showed that the association of amphotericin B and nystatin with AgNPs was extremely effective against *C. parapsilosis* (Figure 2).

In a study of AgNPs and the antifungals fluconazole and itraconazole against *C. albicans*, a strong synergistic effect was observed [18]. However, our results were more expressive, since the studies described above used few microbial strains, whereas 10 strains of *C. parapsilosis* were used in our study. Polyene antifungals act by binding to the plasma membrane of *C. parapsilosis*, which leads to death [4]. AgNPs bind to important cell structures -proteins and DNA- and cause significant cellular damage [16]. The AgNPs and polyene antifungals act in different locations which enhancing the synergistic action. The combination of both substances can have their effects potentiated, therefore showing a large zone of growth inhibition (Figure 2 and 3). Therefore, the *C. parapsilosis* used in this study showed high sensitivity to polyene antifungals when combined with AgNPs.

Conclusion

In summary, our results showed that this new technological product (polyene antifungals and AgNPs) can help fight fungal infections. Other studies with a larger number of strains and toxicity tests should be conducted to know the potential of this combination.

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