Microbes and Infectious Diseases

Original article

Effect of silver nanoparticles on different *Candida* species isolated from patients with oral candidiasis

*Marwa Gamal Bahey *[1], Basant Mostafa Gabr [1], Amr Mostafa Gabr [2], Alaa Mohamed Abo Hagar [3], Eman E. Hegazy [1]*

1-Department of Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt

2- Department of Oral medicine, Periodontology, Oral Diagnosis & Oral Radiology, Faculty of Dentistry, Tanta University

3- Department of Anesthesiology, surgical intensive care and pain medicine, Faculty of Medicine, Tanta University

A R T I C L E I N F O

A B S T R A C T

Background: Oral candidiasis is an opportunistic infection of the oral cavity that can be distributed via the bloodstream, resulting in serious systemic candidiasis. Because of the toxicity and side effects of antifungal agents, research on the antifungal impact of silver nanoparticles (AgNPs) is necessary. **Methods:** Oral swabs were collected from fifty patients with oral candidiasis for the isolation and identification of candida species by automated Vitek 2. Biofilm formation was evaluated using a tissue culture plate assay. The anti-candida and anti-biofilm effects of AgNPs on isolated *Candida species* were investigated using agar-well diffusion and microdilution methods. **Results:** *Candida albicans* was the predominant isolate, accounting for 51.9%. Biofilm formation was identified in 63.5% of candida isolates, commonly in isolates of *C. albicans* (57.6%), *C. tropicalis* (30.3%), and *C. parapsilosis* (12.1%), while all *C. glabarata* isolates could not form a biofilm. A significant decrease in the growth of candida isolates was detected at 500 µg/mL of AgNPs. The largest zone of inhibition (22.6 mm ± 0.6) was detected with *C. albicans*, then *C. parapsilosis* (20.4 mm ± 0.9), *C. tropicalis* (18.4 mm ± 0.5), and *C. glabrata* (16.5 mm ± 0.6) with a statistically significant difference. AgNPs inhibited the biofilm production of *Candida species* up to 100% at 500 µg/ml. Generally, the AgNPs anti-biofilm activity was significantly higher against *C. albicans* than *C. tropicalis* and *C. parapsilosis* at concentrations ranging from 31.25 to 250 µg/ml. **Conclusions:** Synthesized AgNPs showed antifungal and antibiofilm activity against all albicans and non-albicans *Candida species*.

Introduction

Oral candidiasis (OC), also known as thrush, is a frequent opportunistic infection of the oral cavity caused by an overgrowth of different candida species, mostly *Candida albicans,* and an invasion of superficial tissues [1,2].

Moreover, other non-*C.albicans* (NCA) species, such as *Candida tropicalis*, *Candida*

parapsilosis, and *Candida glabrata,* are also involved in this infection [3].

It is widespread and underdiagnosed in older individuals, and it is often preventable with appropriate oral hygiene. Moreover, it can indicate a systemic illness like diabetes [4]. Furthermore, it is a prevalent condition among immune-compromised patients. The infection can propagate

---

* *Corresponding author:* Marwa Gamal Bahey

E-mail address: *marwabahey@gmail.com*

via the bloodstream, resulting in severe systemic candidiasis with high morbidity and mortality [5].

Candida albicans is one of the most widespread opportunistic human fungal pathogens that can be a predisposing factor for invasive fungal infections, ranking as the fourth-prominent source of hospital-acquired infections which has a high mortality rate among critically ill and immune-compromised patients [6,7].

The three classes of antifungal agents that are used to treat fungal infections include azoles, echinocandins, and polyenes [8,9]. Common fungicides such as fluconazole, amphotericin-B, and echinocandins can successfully treat most persistent candida infections; conversely, biofilm-associated infections are challenging to cure with conventional medications. Compared with planktonic cells, Candida biofilms are up to 1000 times more resistant to azoles [10].

Due to rising drug resistance, toxicity, side effects, and drug interactions associated with current antifungal medications, there is an imperative requirement to create new and safe antifungal treatments [11].

Silver nanoparticles (AgNPs) have gathered significant interest due to their potent antimicrobial properties [12,13]. This antibacterial activity is exhibited against both Gram-negative and Gram-positive bacteria. Additionally, when combined with optimum antibiotics, AgNPs exhibit a synergistic effect, enabling lower doses of both agents to be used, thus reducing side effects, and potentially curbing the development of multidrug resistance mechanisms [14,15].

Research has demonstrated that AgNPs are effective against various *Candida species* by inhibiting the growth of yeast cells and affecting several virulence factors, such as the production of biofilms [16]. Additionally, AgNPs were effectively utilized to treat candidiasis [17]. AgNPs were proven to have a strong ability to anchor the *C. albicans* cell wall and then invade it, causing structural changes in the integrity of the plasma membrane [18].

As data concerning the antifungal activity of AgNPs is limited in our hospital, research on the antifungal impact of AgNPs is currently required. Therefore, this study aims to evaluate the effect of AgNPs on *Candida species* isolated from clinical cases with OC at Tanta University Hospitals.

Methods

The cross-sectional study was conducted at the Microbiology and Immunology Department, Faculty of Medicine, Tanta University, in six months between September 2023 and February 2024. The study was conducted following the Declaration of Helsinki and had approval from the Research Ethics Committee at the Faculty of Medicine, Tanta University (approval code: 36264PR72/2/23). Fifty patients were included in the study who were hospitalized in the Emergency Intensive Care Unit (ICU) and from outpatient clinics at Tanta University Hospital. Adult patients presenting with signs and symptoms of oropharyngeal candidiasis were enrolled in the study. They were identified by pseudomembranous raised lesions in the oral mucous membrane. The study excluded any patient without signs of oral candidiasis during the study period. A complete history of participants was recorded. Written, informed consent was provided by all participants.

Phenotypic detection of isolates:

Oral lesions were identified, and samples were taken using sterile swabs. Then, they were sent to the laboratory within 30 minutes for processing. Oral swabs were inoculated on Sabouraud dextrose agar (SDA) (Oxoid UK) with chloramphenicol, and then they were incubated at 37 ℃ for 24–48 h. Identification of fungal isolates was done by white, creamy colonies with a yeast-like odor. Under the microscope, *Candida species* appear as Gram-positive cells with or without pseudohyphae. *Candida species* were identified by automated Vitek 2 Compact (bioMerieux, France), as per the manufacturer's instructions. The confirmed isolated *Candida species* were frozen at -20 °C in brain-heart infusion broth with 20% glycerol and then subcultured for further testing [19].

Phenotypic evaluation of biofilm production in different *Candida* species:

The tissue culture plate (TCP) assay was described as the ideal test for the detection of biofilm. Ten ml of trypticase soy broth (TSB) (Oxoid UK) with 1% glucose was inoculated with a loopful of *Candida species* from a fresh culture on SDA (Oxoid UK). Then, the plate was incubated at 37 °C for 24 hours. Fresh medium was used for dilution of the culture mixture at a percentage of 1:100 to obtain $1x10^6$CFU/ml of *Candida* suspensions before incubation for 24 hours at 37℃. The negative control in the plate was the sterile

broth. The free-floating candida species in the wells were removed four times by washing with 0.2 ml of phosphate buffered saline (PBS) at pH 7.2. Sodium acetate (2%) was used for the fixation of adherent biofilms that were stained with 0.1% crystal violet. Finally, the plate was washed with distilled water and dried completely. The experiment was repeated three times. An ELISA reader (Biotek) was used to record the optical densities (OD) of stained biofilm at the 570nm wavelength that was classified as described by Li et al. [20, 21].

Description of silver nanoparticles:

A stock solution of water-soluble spherical AgNPs (19 ± 5 nm) was measured by transmission electron microscope. It was prepared by Nano Tech, Egypt, with a concentration of 2200 ug/mL. The manufacturer reported that AgNPs have been prepared by the chemical reduction method by using AgNO3 as a source of Ag+ ions, which converts the color of AgNPs into grayish yellow by the reduction of the Ag+ ions.

Characterization of the AgNPs by transmission electron microscopy (TEM):

Transmission electron microscopy measurements were performed to describe the size as well as morphology of nanoparticles, which were prepared by placing a drop of dilute suspension on a carbon-glazed copper grid and allowed to be air-dried at ambient temperature. Then, samples were kept in a desiccator until loaded onto a specimen holder for analysis. Analysis was done on a JEOL JEM-2100 high-resolution transmission electron microscope at a voltage of 200 kV, respectively.

Assessment of the anti-candida effects of AgNPs:

Screening the effects of AgNPs on different *Candida* isolates was investigated using the agar-well diffusion technique. Multiple wells of 6 mm diameter were done on SDA plates (Oxoid UK) that were inoculated with 100 μl of 1×10^6 cfu/ml of (o.5 McFarland) *Candida* suspensions, and then 100 μl of AgNPs with different concentrations ranging from 500 to 31.25 μg/ml were incorporated into the wells. Itraconazole was considered the reference antifungal, and dimethyl sulfoxide (DMSO) was the control. After incubation of the plates for 24-48 hours, different inhibition zones were measured [22].

Determining the MIC and MFC of AgNPs:

The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the anti-fungal agent that can inhibit yeast development. The minimum fungicidal concentration (MFC) was determined to be the lowest concentration that kills 99.99% of the proven yeast. Both were calculated by the microdilution method [23]. AgNPs were processed using a two-fold serial dilution. For each well, 100 μl of Sabaroud dextrose broth (Oxoid UK) of each *Candida* suspension was added to sterile 96-well plates. Also, 100 μL of each concentration of AgNPs (starting from 1,000 μg/ml) was added before covering and incubation of the plates at 37°C for 24 hours. MIC was considered the lowest concentration of the tested anti-fungal that suppressed the visible growth of the fungi. The MFC was detected after subculturing from the wells without visible growth on their plates. However, the MFC was defined as the lowest concentration that did not show any fungal growth on the agar [24].

Assessment of AgNPs effects on the biofilm of different *Candida* species:

Assessment of AgNPs effects on the biofilm of different candida species: All *Candida* isolates that produce biofilm were further evaluated for their ability to form biofilm in the presence of AgNPs by microtitre plate assay, as documented by **Saibabu et al.** [25]. 100 μL of Tryptic soya broth suspension (TSB) with 1% glucose from different *Candida* culture suspensions was inoculated in each well of a 96-well microtitre plate. The plates were kept incubated for attachment of cells at 37◦C for 24 h. Then, phosphate buffered saline (PBS) was used for washing the plates three times to remove the non-attached cells. The culture was diluted 1:100 with fresh broth by adding 200 mL of fresh medium and half MIC, one MIC, and double MIC of AgNPs for all *Candida species*, then incubated at 37◦C for 24 h to see biofilm development. The wells were washed with 0.2 ml of PBS and stained with 0.1% crystal violet for 30 min to detect fixation. Deionized water for washing the plates was used until they were completely dried. Both negative and positive control wells in the plate containing AgNPs without isolate and isolate without AgNPs, respectively, were maintained for each strain. The tissue culture assay was done in triplicates. Optical densities (OD) measurements were calculated by an Elisa reader at 570 nm wavelength. The percentage inhibition of biofilm activity was calculated using the following equation: Biofilm inhibition (%) = 1- (OD of wells processed with AgNPs / absorbance of non-processed wells) × 100. The data are expressed as means ± SD.

Statistical analysis of the data

Data was analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were characterized as numbers and percentages. The Chi-square test was employed to study the association between the categorical variables. For continuous data, they were analyzed for normality by the Shapiro-Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, standard deviation, and median for normally distributed quantitative variables F-test (ANOVA) was used to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons. The significance of the acquired results was judged at the 5% level.

Results

Out of 50 cases with oral candidiasis (OC) involved in this study, 58% were female, and 62% ranged from 40 to 60 years old. 40% of cases were diabetic, 22% had cardiovascular disorders, and 20% of recruited cases were cancer patients, as demonstrated in **table (1)**. Regarding the isolation and identification of fungal isolates, about 52 *Candida species* were isolated. Four different *Candida species* were categorized. *C. albicans* was the most predominant, representing 51.9%, followed by *C. tropicalis* (30.8%), *C. parapsilosis* (9.6%), and *C. glabarata* (7.7%), as demonstrated in **table (2)**.

Regarding the biofilm formation among the studied *candida* isolates, biofilm positivity was detected in 63.5% of *Candida* isolates, most commonly in *C. albicans* isolates (57.6%), followed by *C. tropicalis* (30.3%), and *C. parapsilosis* (12.1%), while all *C. glabarata* isolates could not form a biofilm, as shown in **table (3a)**. Biofilm-former *C. albicans* isolates were classified as strong, moderate, and weak biofilm producers at 47.4%, 42.1%, and 10.5%, respectively. Biofilm-former non-albicans *Candida species* were categorized as strong, moderate, and weak biofilm producers with 35.7%, 64.3%, and 0%, respectively, with no significant statistical difference between albicans and non-albicans species ($p > 0.05$) as reported in **table (3b)**.

Regarding the evaluation of the anti-candidal activity of AgNPs, a significant decrease in the growth of *Candida* was detected at 500 μg/mL of AgNPs. The largest zone of inhibition (22.6 mm ± 0.6) was detected with *C. albicans*, followed by *C. parapsilosis* (20.4 mm ± 0.9), *C. tropicalis* (18.4 mm ± 0.5), and *C. glabrata* (16.5 mm ± 0.6), with a statistically significant difference ($P<0.001$) as demonstrated in **table (4).**

The MIC values of AgNPs against all investigated *Candida species* ranged from 62–250 μg/mL, whereas the MFCs were 125 and 500 μg/mL. The detected MFCs are noticeably higher in comparison to MICs. Regarding *C. albicans*, 13 out of 27 isolates showed MIC at 125 μg/mL and MFC at 250 μg/ml. Additionally , 75% (12/16) of *C. tropicalis* isolates showed MIC and MFC at 250 and 500 μg/mL, respectively. All strains of *C. parapsilosis* and *C. glabrata* showed MICs at 125 μg/mL and MFCs at 250 μg/mL and 500 μg/mL, respectively, as shown in **table (5)**.

As regards the evaluation of the anti-biofilm activity of different concentrations of AgNPs against the isolated *Candida species*, The AgNPs anti-biofilm effect on the tested *Candida* was revealed at a concentration as low as 31.25 μg/L, and total inhibition was detected at 500 μg/mL. AgNPs inhibited the biofilm formation of *Candida* up to 100% at 500 μg/ml. Generally, the AgNPs anti-biofilm activity was significantly higher against *C. albicans* than *C. tropicalis* and *C. parapsilosis* at concentrations from 31.25 to 250 μg/ml ($p <0.001$), as reported in **table (6)** and **Figure 1**.

Table 1. Demographic data and clinical characteristics of the studied cases (n = 50).

	No. (%)
Gender	
Male	21 (42%)
Female	29 (58%)
Age	
<40	7 (14%)
40-60	31 (62%)
≥60	12 (24%)
Underlying conditions	
Cardiovascular diseases	11 (22%)
Diabetes	20 (40%)
Chronic kidney diseases	5 (10%)
Cancer	10 (20%)
Immunological disorders	4 (8%)

Table 2. Distribution of isolated *Candida species* (n = 52).

Candida species	No. (%)
Candida albicans	27 (51.9%)
Non-albicans	
Candida tropicalis	16 (30.8%)
Candida parapsilosis	5 (9.6%)
Candida glabarata	4 (7.7%)

Table 3a. Distribution of the isolated *Candida species* as regard biofilm formation (n = 52)

	Candida albicans	*Candida tropicalis*	*Candida parapsilosis*	*Candida glabarata*	**Total**
Non-biofilm producers	8 (42.1%)	6 (31.6%)	1 (5.3%)	4 (21.1%)	19 (36.5%)
Biofilm producers	**19 (57.6%)**	**10 (30.3%)**	**4 (12.1%)**	**0 (0%)**	**33 (63.5%)**
Strong	9 (47.4%)	4 (40%)	1 (25.0%)	–	14 (42.4%)
Moderate	8 (42.1%)	6 (60%)	3 (75.0%)	–	17 (51.5%)
Weak	2 (10.5%)	0 (0%)	0 (0%)	–	2 (6.1)

Table 3b. Comparison between *C. albicans* and *non-albicans* regard biofilm formation

	Non albicans (n = 25)	*Candida albicans* (n = 27)	χ^2	*p*
Non-biofilm producers	11 (44%)	8 (29.6%)	1.156	0.282
Biofilm producers	**14 (56%)**	**19 (70.4%)**		
Strong	5 (35.7%)	9 (47.4%)		
Moderate	9 (64.3%)	8 (42.1%)		
Weak	0 (0.0%)	2 (10.5%)		

χ^2: Chi square test   MC: Monte Carlo

p: *p* value for comparing *Candida Non albicans* and *Candida albicans*.

*: Statistically significant at $p \leq 0.05$

Table 4. Zone of inhibition of isolated candida species (in mm) at different concentrations of AgNPs in (µg/ml)

Organism		Concentration of AgNPs				
	N	31.25	62.5	125	250	500
Candida albicans	27	6 ± 0	8 ± 1.2	10.2 ± 0.9	14.5 ± 0.8	22.6 ± 0.6
Non albicans Candida spp	25	6.8 ± 1.2	9.04 ± 0.79	11.7 ± 0.99	14 ± 1.06	18.5 ± 1.3
C. tropicalis	16	7.3 ± 1.3	9.5 ± 0.5	11.7 ± 0.5	13.8 ± 0.4	18.4 ± 0.5
C. parapsilosis	5	6 ± 0	8.4 ± 0.55	13 ± 0	15.8 ± 0.4	20.4 ± 0.9
C. glabrata	4	6 ± 0	8 ± 0	10 ± 0	12.6 ± 0.5	16.5 ± 0.6
t(p)		3.562* (0.002*)	3.798* (<0.001*)	5.592* (<0.001*)	1.888 (0.065)	14.176* (<0.001*)

Data was expressed using Mean ± SD   SD: Standard deviation   t: Student t-test

p: *p* value for comparing *Candida albicans* and *non albicans Candida*.

*: Statistically significant at $p \leq 0.05$

Table 5. MIC and MFC for AgNPs against isolated *Candida species* (n = 52).

	Concentration of AgNPs							
	62 (µg/mL)		125 (µg/mL)		250 (µg/mL)		500 (µg/mL)	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans (n)	9	0	13	9	5	13	0	5
Candida tropicalis (n)	0	0	4	0	12	4	0	12
Candida parapsilosis (n)	0	0	5	0	0	5	0	0
Candida glabrata (n)	0	0	4	0	0	0	0	4

Table 6. Comparison between *C. albicans, C. tropicalis* and *C. parapsilosis* according to percentage of biofilm inhibition in different concentrations of AgNPs (n = 33)

		Concentration of AgNPs				
Organism	N	31.25	62.5	125	250	500
C. albicans	19	68.7 ± 4.5	84.1 ± 5.2	97.4 ± 1.7	100 ± 0	100 ± 0
C. tropicalis	10	36.3[a] ± 5.1	50.3[a] ± 3.2	74.1[a] ± 2.3	94.4[a] ± 3.2	100 ± 0
C. parapsilosis	4	58.8[ab] ± 2.5	76.5[ab] ± 1.3	90.8[ab] ± 6.2	100[b] ± 0	100 ± 0
F(p)		168.382[*] (<0.001[*])	192.545[*] (<0.001[*])	246.736[*] (<0.001[*])	36.267[*] (<0.001[*])	–

Data was expressed using Mean ± SD   SD: Standard deviation.

F: F for One way ANOVA test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Tukey)

p: p value for comparing between the *candida albicans, tropical and parapsilosis*

*: Statistically significant at $p \leq 0.05$

a: Significant with *Candida albicans*   b: Significant with *Candida tropicalis*

Figure 1. Percentage of biofilm inhibition in different concentrations of AgNPs.
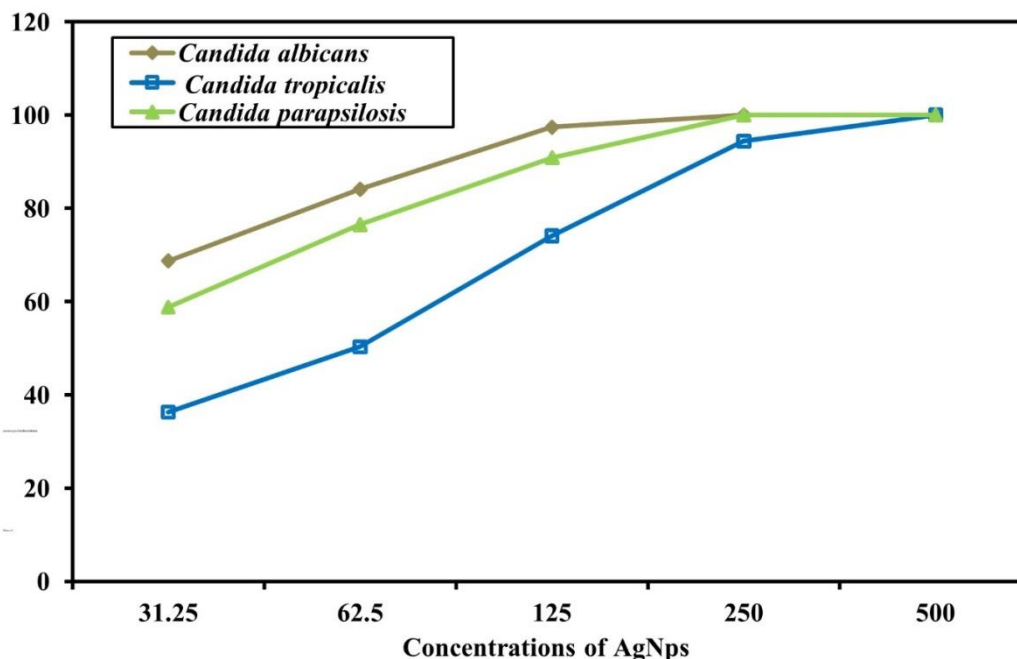
Discussion

Out of 50 patients with oral candidiasis (OC) involved in this study, 58% were female and 62% ranged from 40 to 60 years old. Same findings were reported by another study, which found that the prevalence of OC with diabetes was higher in middle-aged adults (40–60 years) (67%) and among females (65%) [26].

In the current study, the most frequent preexisting disease was diabetes mellitus (40%) followed by cardiovascular disease (22%). Diabetes

mellitus is an essential predisposing factor for OC. This may be related to salivary hypofunction correlated with diabetes mellitus, and the presence of glucose enhances the growth of candida in saliva [4]. However, **da Silva et al.** found that the most common preexisting disease was hypertension (22.7%), followed by cardiovascular disease (13.63%), and diabetes (9.09%) [27]. Moreover, another Egyptian study found oral candidal lesions only in 8% of hospitalized patients; although they had uncontrolled diabetes, and this could be explained as the flourishing of candida is related not only to diabetes but is multifactorial. [28].

In the present study, 20% of the recruited cases were cancer patients with weakened immunity that favors the growth of *candida*. Similar findings were detected in another study that investigated Vietnamese cancer patients, in which 76 *Candida species* were isolated from their oral lesions [29]. The mentioned predisposing factors are involved in the conversion of *Candida species* from a benign commensal state to a pathogenic state. Moreover, the high occurrence of *candida* superinfection is related to the reduction of the normal flora that generates favorable conditions for fungi to proliferate [30].

In the present study, 52 *Candida species* were isolated from 50 samples from patients with OC. Four different *Candida species* were identified. *C. albicans* was the most common *candida* isolate, accounting for 51.9%, which agrees with previous reports [31–33].

Moreover, non-*Candida albicans* species were recorded at 48.07%, as follows: *C.tropicalis* 30.8%, *C. parapsiliosis* 9.6%, and *C. glabarata* 7.7%. Similarly, another study found that 15.4% and 10.3% of non-albicans species with oropharyngeal lesions were *C. tropicalis* and *C. glabarata,* respectively. Unlike our results, they did not find any *C. parapsiliosis* [33].

In the same line, **Nguyen et al.** reported that *C. albicans* remained the most prevalent species, followed by *C. glabrata*, *C. tropicalis*, and *C. krusei* [34]. On the other hand, the current research found that *C. tropicalis* prevalence was ten times higher compared to the previous study by **Salehi et al.** [30].

In the current study, although the predominant *candida* isolates were *C. albicans*, there was a rise in the detection of non-albicans *candida* isolates, which agrees with the previous finding [30, 35].

Generally, *Candida species* can form well-organized biofilms made up of different cell types and even microbial species [36]. In the present study, biofilm positivity was detected in 63.5% of *candida* isolates, most frequently in isolates of *C. albicans* (57.6%), followed by *C. tropicalis* (30.3%) and *C. parapsiliosis* (12.1%), while all *C. glabarata* isolates could not form a biofilm. Biofilms not only shield *Candida species* from antifungal drugs and immunological responses, but they also encourage the development of antifungal-resistant strains, making eradication more difficult [37].

Our study revealed that biofilm-former *C. albicans* isolates were classified as strong, moderate, and weak biofilm producers at 47.4%, 42.1%, and 10.5%, respectively. Biofilm-former non-*albicans Candida species* produced strong biofilm with 35.7%, 64.3%, and 0% strong, moderate, and weak biofilm producers, respectively, with no significant statistical difference between albicans and non-albicans species ($p > 0.05$). Although all isolates were identified from OC lesions, there were variations in the ability of biofilm production by different *Candida species*. There are many other virulence determinants, rather than biofilm production, that help non-biofilm formers to initiate the lesions of OC. Similarly, **Hawser and Douglas** found that isolates of *C. glabrata* and *C parapsilosis* significantly exhibited a lower tendency to form biofilms in comparison to *C. albicans* isolates [38].

In contrast, **Mohandas et al.** found that 51% of the isolated *C. albicans* developed biofilm, which was significantly lower than the percentage of all non-albicans *Candida species* isolates forming biofilm. Strong biofilm formation was detected in *C. tropicalis*. and *C. krusei*, while weak biofilm formation was observed in *C. albicans* [39]. Moreover, Vita´lis et al. reported that all isolated *Candida species* were biofilm formers [40].

While assessing the anti-candida activity of AgNPs, a significant decrease in the growth of *Candida* isolates was perceived at 500 μg/mL of AgNPs. The largest zone of inhibition (22.6 mm ± 0.6) was detected with *C. albicans*, then *C. parapsilosis* (20.4 mm ± 0.9), *C. tropicalis* (18.4 mm ± 0.5), and *C. glabrata* (16.5 mm ± 0.6) with a statistically significant difference. Similar results were recorded by **Jalal et al.** and **Yasir et al.**, who reported a significant decrease in the growth of *Candida species* at the same concentration of AgNPs [41,42]. Antifungal effects were

demonstrated by silver nanoparticles. AgNPs are also useful in curing local infections brought on by *C. tropicalis* and *C. albicans*, as reported by **Li et al.** [43].

The MIC values of AgNPs against all evaluated *Candida* species ranged from 62–250 µg/mL, while the MFCs were 125 and 500 µg/mL. The MFCs that have been identified are significantly more than the MICs. This finding was mentioned before in another study [44].

The findings of the current study declare that the anti-candida activity of AgNPs on the different *candida* species is affected by the dose. The concentration-dependent anti-candida effect of AgNPs has been previously documented by **Jalal et al.** and **Yasir et al.** [41, 42].

Regarding *C. albicans*, 13 out of 27 isolates (48.1%) exhibited MIC at 125 µg/mL and MFC at 250 µg/ml. Additionally , 75% (12/16) of *C. tropicalis* isolates showed MIC and MFC at 250 and 500 µg/mL, respectively. All strains of *C. parapisillosis* and *C. glabrata* showed MICs at 125 µg/mL and MFCs at 250 µg/mL and 500 µg/mL, respectively. These variations in MICs and MFCs of AgNPs on various *Candida species* may be related to different factors, e.g., the shape, size, concentration, and physicochemical properties of nanoparticles [45,46].

Additionally, the diversity in MICs and MFCs could be attributed to the multifaceted character and structural arrangement of the cell walls of each species. Differences were varied between the outer cell wall layer thickness and the structural arrangement of polysaccharide molecules in this layer [47].

Normally, yeast cells colonize and stick to hard or soft surfaces, like those present in the oral cavity, mediated by specific adhesion factors that result in biofilm generation. The AgNPs anti-biofilm effect on the assessed *candida* isolates was revealed at a concentration of 31.25 µg/L, and total inhibition was detected at 500 µg/mL.

In the current study, AgNPs inhibited the biofilm production of *Candida species* up to 100% at 500 µg/ml. Generally, the AgNPs anti-biofilm activity was significantly higher against *C. albicans* than *C. tropicalis* and *C. parapisillosis* at concentrations from 31.25 to 250 µg/ml (*P* <0.001). Our findings showed that the biofilm formed by *C. tropicalis* was the most resistant to the inhibitory effect of AgNPs in comparison with other biofilm-

forming candida species. This finding agreed with other studies, which found that the isolates of *C. tropicalis* were the strongest biofilm producers [48].

Another study revealed the decreased metabolic activity of cells in the *C. tropicalis* biofilm due to the thick extra polysaccharide matrix that may limit the transportation of oxygen and nutrients to these cells [49]. The effective biological activity of AgNPs to penetrate the biofilm is related to the design, size, and concentration of these nanomaterials [50]. Furthermore, other studies revealed that the efficacy of AgNPs against biofilm former *Candida species* is increased when combined with antifungals or antiseptics [51,52].

The inability to compare the anticandidal efficacy of silver nanoparticles with different sizes and designs due to financial constraints was one of the study's limitations. Also, the outcome of this study suggests the application of AgNPs in implant coatings of medical devices to treat resistant fungal infections. However, in vivo studies need to be conducted to detect the bioavailability, mechanism of action, and cytotoxicity of AgNPs before they are employed for biomedical purposes.

Conclusion

The highest prevalence of oral candidiasis was found in diabetic patients, with the greatest number of isolated biofilm-producing *C. albicans*. Synthesized AgNPs showed both antifungal and antibiofilm activity against all albicans and non-albicans *Candida species*. The antifungal activity of AgNPs is concentration-dependent. Overall, the AgNPs could be employed as a promising alternative topical therapy for OC in high-risk patients.

Financial disclosure. None.

Conflict of interest: No hidden conflicts of interest for the writers to mention.

References

1- **Hellstein JW, Marek CL.** Candidiasis: red and white manifestations in the oral cavity. Head and neck Pathology. 2019; 13:25-32.

2- **Millsop JW, Fazel N.** Oral candidiasis. Clinics in dermatology. 2016 ;34(4):487-94.

3- **Savastano C, Silva ED, Gonçalves LL, Nery JM, Silva NC, Dias AL.** Candida glabrata among Candida spp. from environmental health

practitioners of a Brazilian Hospital. brazilian journal of microbiology. 2016; 47:367-72.

4- **Akpan A, Morgan R.** Oral candidiasis. Postgraduate medical journal. 2002;78(922):455-9.

5- **Telles DR, Karki N, Marshall MW.** Oral fungal infections: diagnosis and management. Dental Clinics. 2017;61(2):319-49.

6- **Geffers C, Gastmeier P.** Nosocomial infections, and multidrug-resistant organisms in Germany: epidemiological data from KISS (the Hospital Infection Surveillance System). Deutsches Ärzteblatt International. 2011;108(6):87.

7- **Rezaie S, Zareshahrabadi Z, Zomorodian K, Pakshir K, Mehrabani D, Nouraei H, et al**. Morphogenesis and pathogenesis regulation of Candida albicans by probiotic bacterium "Pediococcus acidilactic. Journal of Microbiology, Biotechnology and Food Sciences. 2020 ;10(1):5-11.

8- **Bertolini M, Dongari-Bagtzoglou A.** The relationship of Candida albicans with the oral bacterial microbiome in health and disease. InOral mucosal immunity and microbiome 2019 (pp. 69-78). Springer, Cham.

9- **Ahmad S, Joseph L, Parker JE, Asadzadeh M, Kelly SL, Meis JF, et al.** ERG6 and ERG2 are major targets conferring reduced susceptibility to amphotericin B in clinical Candida glabrata isolates in Kuwait. Antimicrobial Agents and Chemotherapy. 2019 ;63(2):10-128.

10- **Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ.** Invasive Candidiasis. Nature Reviews Disease Primers. 2018;4(1):1-20.

11- **Huang T, Li X, Maier M, O'Brien-Simpson NM, Heath DE, O'Connor AJ.** Using inorganic nanoparticles to fight fungal infections in the antimicrobial resistant era. Acta Biomaterialia. 2023 ;158: 56-79.

12- **Zhu C, Liao B, Ye X, Zhou Y, Chen X, Liao M, et al.** Artemisinin elevates ergosterol levels of Candida albicans to synergise with amphotericin B against oral candidiasis. International journal of antimicrobial agents. 2021; Sep 1;58(3):106394.

13- **Van Daele R, Spriet I, Wauters J, Maertens J, Mercier T, Van Hecke S, et al.** Antifungal drugs: what brings the future. Medical mycology. 2019; Jun 1;57(Supplement_3): S328-43.

14- **Liao S, Zhang Y, Pan X, Zhu F, Jiang C, Liu Q et al.** Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant Pseudomonas aeruginosa. Int J Nanomedicine 2019; 14:1469-1487.

15- **Surwade P, Ghildyal C, Weikel C, Luxton T, Peloquin D, Fan, X et al.** Augmented antibacterial activity of ampicillin with silver nanoparticles against methicillin-resistant Staphylococcus aureus (MRSA). The Journal of Antibiotics 2019; 72(1): 50-53

16- **Ahamad I, Bano F, Anwer R, Srivastava P, Kumar R, Fatma T.** Antibiofilm activities of biogenic silver nanoparticles against Candida albicans. Frontiers in Microbiology. 2022; 12:741493.

17- **Ali EM, Abdallah BM.** Effective inhibition of candidiasis using an eco-friendly leaf extract of calotropis-gigantean-mediated silver nanoparticles. Nanomaterials. 2020 ;10(3):422.

18- **Siddiqi KS, Husen A, Rao RA.** A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of nanobiotechnology. 201;16(1):1-28.

19- **Murray CK, Beckius ML, Green JA, Hospenthal DR.** Use of chromogenic medium

in the isolation of yeasts from clinical specimens. Journal of medical microbiology. (2005); 54(10), 981-985.

20- **Panda PS, Chaudhary U, Dube SK.** Comparison of four different methods for detection of biofilm formation by uropathogens. Indian Journal of Pathology and Microbiology. 2016; 59: 177- 179.

21- **Li X, Yan Z, Xu J.** Quantitative variation of biofilms among strains in natural populations of Candida albicans. Microbiology 149.2 .2003; 353-362.

22- **Jalal M, Ansari MA, Shukla AK, Ali SG, Khan HM, Pal R, et al.** Green synthesis and antifungal activity of Al 2 O 3 NPs against fluconazole-resistant Candida spp isolated from a tertiary care hospital. RSC advances. 2016;6(109):107577-90.

23- **Abed-Alwahed FR, Al-Baqi NJA.** Effect of the fluconazole resistant erg11expression in fluconazole-resistant Candida albicans isolates. Plant Arch.2020; 20, 616-620.

24- **Arsène MM, Podoprigora IV, Davares AK, Razan M, Das MS, Senyagin AN.** Antibacterial activity of grapefruit peel extracts and green-synthesized silver nanoparticles. Veterinary World. 2021;14(5):1330.

25- **Saibabu V, Fatima Z, Ahmad K, Khan LA, Hameed S.** Octyl gallate triggers dysfunctional mitochondria leading to ROS driven membrane damage and metabolic inflexibility along with attenuated virulence in Candida albicans. Medical mycology. 2020;58(3):380-92.

26- **Moustafa EE, Kheiralla ZM, Maklad SS, Ashour SM, Raoof MA.** Prevalence of oral Candidiasis and Risk Factors in Diabetic Patients. Journal of Scientific Research in Science. 2015;32(part 1):58-77.

27- **da Silva NP, Borges-Paluch LR, dos Santos Cerqueira TP, Nova MX, de Almeida Costa TB, de Jacobi CC.** Prevalence of Candida in oral cavity of patients wearing dental prosthesis. MUNDO DA SAUDE. 2015;39(3):325-32.

28- **Shaheen MA, Taha M.** Species Identification of Candida Isolates Obtained from Oral lesions of Hospitalized and Non-Hospitalized Patients Zith Oral Candidiasis. Egyptian dermatology online journal. 2006 Jun 14;2(1):1.

29- **Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA.** Oral candidiasis: a disease of opportunity. Journal of Fungi. 2020; Jan 16;6(1):15.

30- **Salehi M, Ahmadikia K, Mahmoudi S, Kalantari S, Jamalimoghadamsiahkali S, Izadi A, et al.** Oropharyngeal candidiasis in hospitalized COVID-19 patients from Iran: Species identification and antifungal susceptibility pattern. Mycoses. 2020;63(8):771-8.

31- **Kwamin F, Nartey NO, Codjoe FS, Newman MJ.** Distribution of Candida species among HIV-positive patients with oropharyngeal candidiasis in Accra, Ghana. The Journal of Infection in Developing Countries. 2013;7(01):041-5.

32- **Yu SY, Zhang L, Chen S, Kong F, Xiao M, Wang H, et al.** Candida isolates causing refractory or recurrent oropharyngeal candidiasis in 11 hospitals in China. Infection and Drug Resistance. 2019:865-75.

33- **Khalil MA, El-Ansary MR, Bassyouni RH, Mahmoud EE, Ali IA, Ahmed TI, et al.** Oropharyngeal candidiasis among Egyptian COVID-19 patients: Clinical characteristics, species identification, and antifungal susceptibility, with disease severity and fungal

coinfection prediction models. Diagnostics. 2022;12(7):1719.

34- **Nguyen BV, Nguyen HH, Vo TH, Le MT, Tran-Nguyen VK, Nguyen PV.** Prevalence and drug susceptibility of clinical Candida species in nasopharyngeal cancer patients in Vietnam. One Health. 2024; 18:100659.

35- **Awad SM, Elbrolosy AM, Salem EH, Abdallah HM, Amin M.** Candidiasis in Immunnocompromised Patients; Comparison between C. albicans and Non-albicans regarding the Type of Infection, Biofilm Formation and Virulence Genetic Profile. Egyptian Journal of Medical Microbiology. 2019;28(2):157-66.

36- **Polke M, Hube B, Jacobsen ID.** Candida survival strategies. Advances in Applied Microbiology. 2015; 91:139-235.

37- **Ansari MA, Kalam A, Al-Sehemi AG, Alomary MN, AlYahya S, Aziz MK, et al.** Counteraction of biofilm formation and antimicrobial potential of Terminalia catappa functionalized silver nanoparticles against Candida albicans and multidrug-resistant Gram-negative and Gram-positive bacteria. Antibiotics. 2021;10(6):725.

38- **Hawser SP, Douglas LJ.** Biofilm formation by Candida species on the surface of catheter materials in vitro. Infection and immunity. 1994;62(3):915-21.

39- **Mohandas V, Ballal M.** Distribution of Candida species in different clinical samples and their virulence: biofilm formation, proteinase, and phospholipase production: a study on hospitalized patients in southern India. Journal of global infectious diseases. 2011;3(1):4-8.

40- **Vitális E, Nagy F, Tóth Z, Forgács L, Bozó A, Kardos G, et al** Candida biofilm production is associated with higher mortality

in patients with candidaemia. Mycoses. 2020;63(4):352-60.

41- **Jalal M, Ansari MA, Alzohairy MA, Ali SG, Khan HM, Almatroudi A, Siddiqui MI.** Anticandidal activity of biosynthesized silver nanoparticles: effect on growth, cell morphology, and key virulence attributes of Candida species. International journal of nanomedicine. 2019; 14:4667.

42- **Yasir M, Singh J, Tripathi MK, Singh P, Shrivastava R.** Green synthesis of silver nanoparticles using leaf extract of common arrowhead houseplant and its anticandidal activity. Pharmacognosy Magazine. 2017 Oct;13(Suppl 4):S840.

43- **Li C, Wang X, Chen F, Zhang C, Zhi X, Wang K, et al.** The antifungal activity of graphene oxide-silver nanocomposites. Biomaterials. 2013;34(15):3882–90.

44- **Panáček A, Kolář M, Večeřová R, Prucek R, Soukupová J, Kryštof V, et al.** Antifungal activity of silver nanoparticles against Candida spp. Biomaterials. 2009;30(31):6333-40.

45- **Barapatre A, Aadil KR, Jha H.** Synergistic antibacterial and antibiofilm activity of silver nanoparticles biosynthesized by lignin-degrading fungus. Bioresources and Bioprocessing. 2016; 3:1-3.

46- **Muthamil S, Devi VA, Balasubramaniam B, Balamurugan K, Pandian SK.** Green synthesized silver nanoparticles demonstrating enhanced in vitro and in vivo antibiofilm activity against Candida spp. Journal of basic microbiology. 2018;58(4):343-57.

47- **Khatoon N, Mishra A, Alam H, Manzoor N, Sardar M**. Biosynthesis, characterization, and antifungal activity of the silver nanoparticles against pathogenic Candida species. BioNanoScience. 2015; 5:65-74.

48- **Zuza-Alves DL, Silva-Rocha WP, Chaves GM.** An update on Candida tropicalis based on basic and clinical approaches. Frontiers in microbiology. 2017; 8:281220.

49- **Alnuaimi AD, O'Brien-Simpson NM, Reynolds EC, McCullough MJ.** Clinical isolates and laboratory reference Candida species and strains have varying abilities to form biofilms. FEMS yeast research. 2013;13(7):689-99.

50- **Szerencsés B, Igaz N, Tóbiás Á, Prucsi Z, Rónavári A, Bélteky P, et al.** Size-dependent activity of silver nanoparticles on the morphological switch and biofilm formation of opportunistic pathogenic yeasts. BMC microbiology. 2020; 20:1-3.

51- **Monteiro DR, Silva S, Negri M, Gorup LF, de Camargo ER, Oliveira R, et al.** Antifungal activity of silver nanoparticles in combination with nystatin and chlorhexidine digluconate against Candida albicans and Candida glabrata biofilms. Mycoses. 2013;56(6):672–80.

52- **Silva S, Pires P, Monteiro DR, Negri M, Gorup LF, Camargo ER, et al.** The effect of silver nanoparticles and nystatin on mixed biofilms of Candida glabrata and Candida albicans on acrylic. Med Mycol. 2013;51(2):178–84.