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ANTIFUNGAL SUSCEPTIBILITY AND COMPARATIVE ANALYSIS OF BIOFILM PRODUCTION AND METABOLIC ACTIVITY IN *CANDIDA* SPP. ISOLATED FROM CLINICAL SAMPLES

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Candida, known as a commensal yeast in the oral and vaginal canals, can also cause a range of opportunistic diseases. Its ability to form biofilms is what underlies its infectious pathogenicity. This research aimed to examine clinical Candida isolates regarding their ability to form biofilms and their susceptibility to antifungal drugs. Samples from vaginal and oral sites were collected and identified using phenotypic tests such as CHROMagar, and carbohydrate assimilation. Total biofilm biomass and metabolic activity were assessed using crystal violet staining and tetrazolium salt reduction, respectively. Out of 120 Candida species isolated, C. albicans was the most common (85%), followed by C. glabrata (12.5%), C. dubliniensis (1.67%), and C. tropicalis (0.83%). The majority of isolates showed high sensitivity to fluconazole (93.3%), followed by amphotericin B (79.2%). A significant correlation was observed between biofilm biomass and metabolic activity, with non-albicans Candida strains exhibiting the highest biofilm formation, followed by C. albicans strains. These findings emphasize the importance of considering these factors when managing Candida infections.

Keywords: *Biofilm biomass, metabolic activity, Candida albicans, non-albicans Candida*

INTRODUCTION

Candida species are ubiquitous fungi, found on mucous membrane surfaces, including the gastrointestinal and genital tracts of healthy individuals. These opportunistic organisms can cause a range of ailments in vulnerable individuals such as the elderly, hospitalized patients, or those with weakened immune systems[1]. *Candida*-related fungal infections can vary from superficial mucocutaneous proliferations to invasive conditions affecting multiple organs [2]. Accurate identification of *Candida* species is crucial for appropriate treatment and management of candidiasis. Various methods, including phenotypic techniques like chromogenic media and carbohydrate assimilation, as well as molecular methods such as polymerase chain reaction (PCR), have

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been employed for characterizing *Candida* species [3]. *Candida albicans* is typically the predominant species responsible for these infections, although there has been a rise in non-*albicans Candida* species like *Candida glabrata*, *C. krusei*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, *C. guilliermondii*, and *C. kefyr* in recent years. Vaginal and oral candidiasis are the most common, with approximately 90% of oral candidiasis cases attributed to these species, and prevalence ranging from 22.5% to 83.3% in children worldwide. Genital candidiasis affects about 75% of women at least once in their lifetime, with recurrence rates estimated between 5% and 8% [1,4,5]. The increased prevalence of these isolated *Candida* species is linked to excessive use of antifungal drugs, leading to resistance, and the rise in patients undergoing immunosuppressive therapy [6,7].

The formation of biofilms in *Candida* species is one of the principal virulence factors promoting the development and persistence of candidiasis. These biofilms have a complex three-dimensional structure providing a protective environment for microorganisms. The biofilm formation process includes initial adhesion, intermediate multiplication with extracellular matrix production, maturation, and dispersion phases. Unfortunately, biofilms often confer resistance to standard antibiotic treatments, making subsequent infections challenging to treat [8,9]. Moreover, different *Candida* species show variations in biofilm-forming ability and sensitivity to antifungal agents. This study aims to identify clinical *Candida* isolates from oral and vaginal mucosa, assess their biofilm-forming capacity, and examine their sensitivity to antifungal agents.

MATERIAL AND METHODS

Clinical isolates

A total of 155 cases with vaginal, and oral thrush symptoms were studied over a one-year period (May 2021 to April 2022). These patients sought medical consultation at Youcef Damardji Hospital in Tiaret, Algeria, where clinical assessments were conducted by specialized doctors. *Candida* was isolated by rubbing a sterile swab across the candidal lesion on the vaginal or oral mucosa. Subsequently, the samples were immediately cultured on sabouraud chloramphenicol (SC) medium, with and without actidione and then incubated at 37 ºC for 48 h. After confirming that the colonies belonged to the *Candida* species, the isolates were purified through successive plating until a pure isolated colony was obtained.

Phenotypic identification of *Candida* isolates

Chromogenic agar culture

Chromogenic media are utilized to differentiate various *Candida* species based on the color of their colonies [10]. Pure yeast isolates were cultured on CHROMagar *Candida* (Realab, Tizi-ouzou, Algeria) and incubated for 48 h at 37 °C. According to the manufacturer's instructions, colonies of *C. albicans* or *C. dubliniensis* should show up green, metallic blue for *C. tropicalis*, pale rose for *C. krusei* and pink for *C. glabrata*.

Germ tube formation test

The purpose of this test was to look for germ tube formation, a characteristic observed only by *C. albicans* and *C. dubliniensis* [11]. One to two well-defined colonies were inoculated into 0.5 ml of fresh human serum and incubated for 3 h at 37 °C. Following incubation, a drop of the suspension was placed between a slide and coverslip and examined under microscope to determine the presence of germ tubes [12,13].

Chlamydospore formation test

The principle of this technique was to inoculate a *Candida* colony onto a nutrient-poor medium in order to observe the formation of resistance spores that distinguish strains of *C. dubliniensis*, *C. albicans* and very infrequently *C. tropicalis* [14]. On rice agar tween (RAT) (Realab, Tizi-ouzou, Algeria) medium, a drop of previous preparation (serum + inoculum) was inoculated and covered with a coverslip. After 48 h of incubation at 37 °C, observations were made directly under microscope.

Carbohydrate assimilation test

Differences in carbohydrate assimilation capacity between species are utilized for their determination. This method relies on the species' ability to assimilate specific sugars as their sole source of carbon [14]. Furthermore,

several studies have shown that *C. dubliniensis* can be differentiated from *C. albicans* by its inability to assimilate D-xylose [15,16]. According to Khan et al. [16], a basal medium promoting yeast growth in the presence of appropriate substrates was prepared with 6.7 g of yeast nitrogen base, 10 g of carbon sources, and 20 g of agar. Yeast suspension was prepared, adjusted to an optical density of 0.15 and then seeded onto the medium. Each plate was inoculated with 8 isolates and incubated for 48h at 37 °C. Carbohydrate assimilation was indicated by yeast growth around the corresponding seeding site.

C. albicans ATCC10237 and *C. albicans* ATCC10231 served as reference strains for all tests.

In-vitro antifungal susceptibility

Candida spp. were tested for susceptibility to antifungal drugs using the disk diffusion method following the Clinical Laboratory Standards Institute (CLSI) M44-A guidelines [17]. After obtaining a turbidity standard of 0.5 McFarland, the fungal solution was inoculated on Mueller Hinton agar supplemented with 0.5 µg/mL methylene blue and 2% glucose, and allowed to dry for 5 to 15 minutes. Subsequently, amphotericin B (20 µg) and fluconazole (25 µg) discs were placed onto the agar surface. Following incubation for 24 h at 37 °C, the zones of inhibition around the discs were measured and interpreted according to the predefined criteria: susceptible (S) for fluconazole at zone diameters of 19 mm and amphotericin B at 11 mm; susceptible dose-dependent (SDD) for fluconazole at zone diameters of 15 to 18 mm; and resistant (R) for fluconazole at zone diameters of 14 mm and amphotericin B at zone diameters of 10 mm [18].

Biofilm assay

The ability of *Candida* species to form biofilms was evaluated using the microtiter plate method. Pure colonies of *C. albicans* and *Candida* non-*albicans* were suspended in yeast peptone dextrose broth (YPD) and cultured for 18 h at 37 °C. The cell density was adjusted to 1-1.5×10^6 CFU/ml using spectrophotometry [19]. Biofilms were formed by transferring 100 µl of standardized cell suspensions into selected wells of round bottom microplates and incubating for 48 h at 37 °C. Each strain was introduced into three wells, and at least six wells were reserved as negative controls (only broth). After biofilm formation, the medium was aspirated and non-adherent cells were removed by washing three times with sterile phosphate buffered saline (PBS).

Measurement of biofilm formation

The biomass and metabolic activity of the formed biofilms were assessed using two different approaches.

Crystal violet assay

The crystal violet (CV) test was used to quantify biofilm formation as previously described, with some modifications [20]. Briefly, 100 µl of Gentian violet was applied for 20 min, followed by four washes with PBS. The dye-bound biofilm was then removed using 100 µl of 95% ethanol, and optical density (OD) was measured at 630 nm for each well using a microplate reader (Maé SAS, France).

TTC reduction assay

The 2,3,5-triphenyltetrazolium chloride (TTC) reduction test was performed to measure the biofilm metabolic activity as previously described with some adjustments [21]. Briefly, 200 µl of YPD broth, supplemented with TTC (Sigma-Aldrich) at a final concentration of 0.05% (w/v) was added to each well and incubated at 37 °C overnight. Following incubation, the plates were rinsed three times with PBS. Subsequently, methanol (96%) was used to remove the red-colored TTC visible in the wells. the OD of the wells was measured at 490 nm.

Biofilms were divided into four categories **(Table 1)** based on the optical density cut-off value (ODc) which were determined as follows:

ODc = average OD of negative control + (3 × standard deviation (SD) of negative control).

All tests were carried out in three replicates, and the average OD values were estimated [22,23].

Statistical Analysis

A statistical analysis was completed with the support of the SPSS program (version 26). Chi-square test was used to compare

percentages significantly between groups. Mann-Whitney test was used to compare differences values of biomass and the metabolic activity of biofilms between the *Candida* groups. The correlation between the CV test and the TTC test was examined using Spearman's rank correlation.

Table 1: Categories of formed biofilms.

OD values' average	Biofilm production
$OD \leq OD_C$	Lack
$OD_C \leq OD \geq 2 \times OD_C$	Weak
$2 \times OD_C \leq OD \geq 4 \times OD_C$	Moderate
$4 \times OD_C \leq OD$	Strong

RESULTS AND DISCUSSION

Results

Identification of *Candida* species from oral and vaginal isolates

In this study, 155 clinical specimens were evaluated, of which 14 were isolated from an oral swab and 141 from a vaginal swab. Most patients in the cases of oral thrush were children, ranging in age from five months to three years. In cases of vaginal thrush, the average age of the patients was 40 years (range 19 – 64 years); women receiving therapy were spared. After culturing on sabouraud-chloramphenicol agar, 120 cultures screened positive for *Candida* (77.42%), while 35 (22.58%) of samples showed no candidal growth **(Table 2)**. There was no significant difference between the positive and negative results of *Candida* strains from oral and vaginal swabs ($P > 0.05$).

Distribution of *Candida* spp. isolated from patients with oral and vaginal candidiasis

As demonstrated in **Table 3**, a variety of *Candida* species were identified in the clinical samples. Out of the 120 cultures analyzed, 102 tested positive for *C. albicans* (85%). Additionally, 18 isolates were classified as non-*albicans* yeasts, with *C. glabrata* representing 12.5% of cases, *C. dubliniensis* 1.67%, and *C. tropicalis* 0.83%.

Table 2: Positive and negative results of *Candida* species from oral and vaginal swabs.

***Candida* spp.**	**Oral swab no. (%)**	**Vaginal swab no. (%)**	**Total no. (%)**
Positive	12 (85.71)	108 (76.60)	120 (77.42)
Negative	2 (14.29)	33 (23.40)	35 (22.58)
Total no.	14	141	155 (100)
Chi-square $-\chi 2$	0.606	0.606	-
P-value	> 0.05	> 0.05	-

Table 3: Distribution of *Candida* spp. in oral and vaginal swabs.

***Candida* spp.**	**Oral swab no. (%)**	**Vaginal swab no. (%)**	**Total no. (%)**
C. albicans	12 (100)	90 (83.33)	102 (85)
C. glabrata	0 (0)	15 (13.89)	15 (12.5)
C. dubliniensis	0 (0)	2 (1.85)	2 (1.67)
C. tropicalis	0 (0)	1 (0.93)	1 (0.83)
Total no.	12 (100)	108 (100)	120 (100)

Antifungal susceptibility test

Table 4 displayed the in vitro sensitivity profiles of *Candida* spp. to fluconazole and amphotericin B. As indicated in the table, the majority of yeast isolates showed sensitivity to both antifungal agents, with fluconazole being the most effective. Out of the 120 isolates, 112 (93.3%) were sensitive to fluconazole, 4 (3.3%) showed intermediate sensitivity and 4 (3.3%) resistance. This was followed by amphotericin B, with 95 isolates (79.2%) displaying susceptibility and 25 (20.8%) exhibiting resistance.

Biofilm forming of *Candida* spp

Table 5 demonstrated the metabolic activity and biomass of biofilms formed for each species using the tetrazolium reduction and crystal violet staining methods, respectively. The biofilm biomass recorded for *C. albicans* species showed a remarkable variation, with ODs ranging from 0.055 to 1.605, which is the cause of the significant standard deviation. Similar findings were noted for metabolic activity, with values ranging from 0.015 to 0.561. For both procedures, the analysis revealed significant differences between the groups ($P < 0.05$). When compared to *C. albicans*, the biofilm biomass produced by *C. glabrata*, and *C. tropicalis* was higher. *C. dubliniensis,* with a DO of 0.148, exhibited the least significant production. The metabolic activity rates varied greatly among the species, with *C. glabrata* having the highest rate with an OD of 0.141. A significant correlation was noted between the biomass of biofilm and the metabolic activity in both *C. albicans* ($r = 0.806$, $P < 0.001$) and *Candida* non-*albicans* strains ($r = 0.645$, $P = 0.004$).

Table 4: In vitro antifungal susceptibility of *Candida* isolates by disk diffusion method.

***Candida* strains**		**No.**	**Antifungal agents *no.* (%)**						
			Fluconazole (25 µg)				**Amphotericin B (20 µg)**		
			Range (mm) [a]	S	SDD	R	Range (mm) [a]	S	R
Vaginal swab	*C. albicans*	90	14.3 – 42.7	85 (94.4)	3 (3.3)	2 (2.2)	9.0 – 22.3	70 (77.8)	20 (22.2)
	C. dubliniensis	2	13.3 – 26.3	1 (50)	0 (0)	1 (50)	18.7 – 19.7	2 (100)	0 (0)
	C. tropicalis	1	30,3	1 (100)	0 (0)	0 (0)	19,7	1 (100)	0 (0)
	C. glabrata	15	18.7 – 27.7	14 (93.3)	1 (6.7)	0 (0)	9.0 – 15.3	11 (73.3)	4 (26.7)
Oral swab	*C. albicans*	12	13.7 – 49.3	11 (91.7)	0 (0)	1 (8.3)	9.7 – 18.7	11 (91.7)	1 (8.3)
Total no. (%)		120	13.3 – 49.3	112 (93.3)	4 (3.3)	4 (3.3)	9.0 – 22.3	95 (79.2)	25 (20.8)
Ref. Strains	*C. albicans* ATCC10231	-	28.3	-	-	-	12.0	-	-
	C. albicans ATCC10237	-	17.3	-	-	-	15.0	-	-

[a] Diameter of zone of inhibition (mean values); R: Resistant; S: Sensible; SDD: Susceptible dose dependent.

Table 5: *Candida* spp. biofilm development as measured by the two methods of TTC reduction and CV staining; Data are based on values obtained by optical density and expressed as mean ± SD and range.

***Candida* spp.**	**No.**	**OD/Mean ± SD (range)**	
		TTC assay	**CV assay**
C. albicans	102	0,106 ± 0,08 (0,015-0,561)	0,165 ± 0,19 (0,055-1,605)
C. non-*albicans*	18	0,139 ± 0,066 (0,035-0,236)	0,250 ± 0,139 (0,074-0,594)
P-Value [a]	-	$P = 0.023$	$P = 0.009$
C. dubliniensis	2	0,137 ± 0,086 (0,076-0,197)	0,148 ± 0,011 (0,140-0,155)
C. tropicalis	1	0,112	0,234
C. glabrata	15	0,141 ± 0,069 (0,035-0,236)	0,264 ± 0,185 (0,074-0,594)
C. albicans ATCC10231	-	0.077 ± 0.011	0.134 ± 0.005
C. albicans ATCC10237	-	0.146 ± 0.030	0.165 ± 0.005

[a] Comparison between groups (*Candida albicans* / *Candida* non-*albicans*).

Biofilm categories of *Candida* spp

Fig. 1 represented the percentage of biofilm categories generated by each species as a function of cut-off optical density values: CV staining test (lack biofilm formation (LBF) OD ≤ 0.066, weak biofilm formation (WBF) 0.066 < OD ≤ 0.133, moderate biofilm formation (MBF) 0.133 < OD ≤ 0.265, high biofilm formation (HBF) OD > 0.265); and TTC reduction test (lack metabolic activity (LMA) OD ≤ 0.050, weak metabolic activity (WMA) 0.050 < OD ≤ 0.10, moderate metabolic activity (MMA) 0.10 < OD ≤ 0.201, high metabolic activity (HMA) OD > 0.201). According to the CV test, 51% of *C. albicans* strains formed a weak biofilm, while the TTC method revealed that 39% of these strains showed low metabolic activity. Among *C. glabrata* strains, 33% exhibited intense biofilm formation as measured by the CV test, followed by *C. albicans* with a percentage of 11%. A moderate biofilm was observed in *C. dubliniensis* and *C. tropicalis*.

Assessment of biofilm-forming ability in resistant *Candida* strains

Table 6 illustrated the biofilm formation status among strains resistant to amphotericin B and fluconazole. Results are differentiated between *C. albicans* and non-*albicans* species. For amphotericin B-resistant *C. albicans* strains, out of a total of 21 strains, 85.7% exhibited biofilm formation, with 38.1% characterized by intense biofilm. Among non-*albicans* species, all strains resistant to amphotericin B formed biofilm, with 50% exhibiting intense biofilm. For fluconazole-resistant strains, biofilm formation was observed in 33.3% of *Candida albicans* strains, while all non-*albicans* species showed biofilm formation. Notably, intense biofilm formation was not observed within this group.

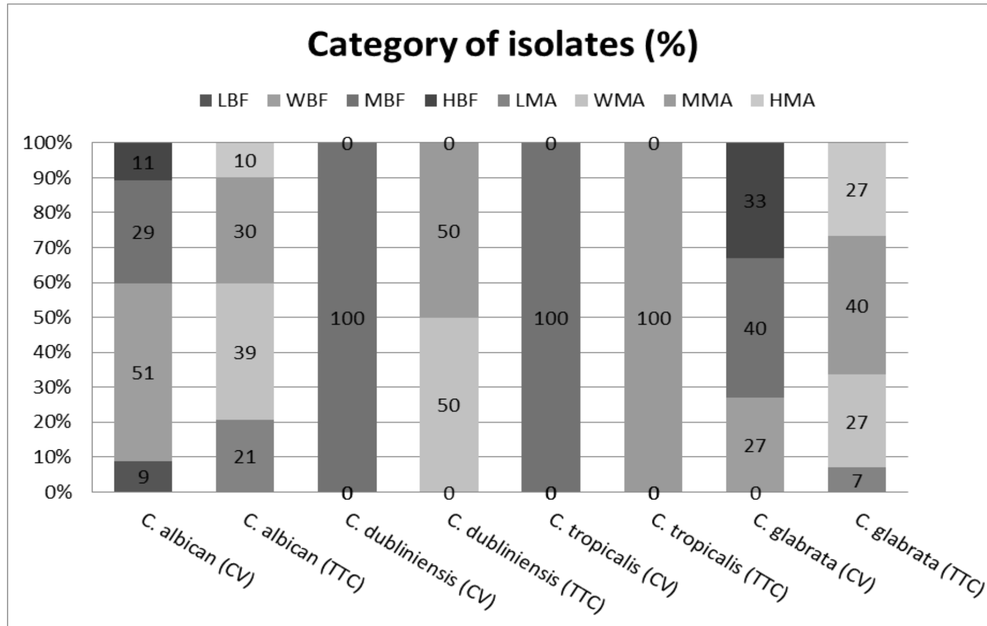

Fig. 1: Percentages of biofilm categories formed by each species as evaluated by the two methods, CV staining and TTC reduction. LBF: lack biofilm formation; WBF: weak biofilm formation; MBF: moderate biofilm formation; HBF: high biofilm formation; LMA: lack metabolic activity; WMA: weak metabolic activity; MMA: moderate metabolic activity; HMA: high metabolic activity.

Table 6: Biofilm formation status in amphotericin B and fluconazole-resistant *Candida* species.

Candida spp.	Number	Strains *no.* (%)	
		Biofilm former	Hight biofilm former
C. albicans AMP-R[a]	21	18 (85.7)	8 (38.1)
Candida non *albicans* AMP-R	4	4 (100)	2 (50)
C. albicans FLC-R[b]	3	1 (33.3)	0 (0)
Candida non *albicans* FLC-R	1	1 (100)	0 (0)

[a] AMP-R: Amphotericin B-resistant.
[b] FLC-R: Fluconazole-resistant.

Discussion

The objective of this study was to assess the distribution of *Candida* strains in Tiaret, Algeria, as well as their susceptibility to two antifungal agents and ability to form biofilms. In the course of our investigation, we analyzed 155 isolates obtained from patients. Based on cultural and microscopic characteristics, all *Candida* isolates exhibited robust growth on SC media after 24 to 48 h of incubation at 37 °C. Conventional methods such as the germ tube test, chlamydospore production, and sugar assimilation were employed to identify *Candida* species. In addition to these traditional tests, a chromogenic medium (CHROMagar *Candida*) was utilized. This medium is distinguished by its capacity to simplify and accelerate the identification procedure by exploiting chromogenicity to differentiate *Candida* species based on biochemical characteristics [10]. However, it should be noted that CHROMagar medium, although a valuable tool, may exhibit reduced reliability in identifying certain species. On this medium, pink colonies can be observed, suggesting the possibility of *C. glabrata* or *C. krusei*. Discrimination between these two species is based primarily on the formation of pseudohyphae, which are present in *C. krusei* but not in *C. glabrata* [10]. Similarly, differentiating *C. albicans* from *C. dubliniensis* on this medium can pose challenges, as both can yield green colonies. However, an additional distinguishing factor lies in their xylose assimilation capacity, where *C. albicans* demonstrates xylose uptake while for the most part *C. dubliniensis* does not [15,16].

Our findings show that *C. albicans* predominates in cases of vaginal candidiasis, accounting for 83.33% of the isolates investigated. This high prevalence of *C. albicans* is consistent with previous research that identified this species as the most common cause of genital fungal infections [24,25]. However, several studies in this field have reported divergent distributions of *C. albicans* prevalence compared to our findings. Notably, Hashemi et al. [26] identified 55.78% non-*albicans Candida*, with *C. lusitaniae* being the most predominant species. while a study reported by Kalaiarasan et al. [27] highlighted *C. glabrata* (45.1%) as the most prevalent of *Candida* species in their findings. Indeed, in recent years, non-*albicans Candida* species have dominated the distribution of *Candida* species in the context of vaginal candidiasis. This incidence may be attributed to factors such as geography, the investigated population, and numerous clinical and physiological variables [28,29]. In oral candidiasis, our research indicates that all isolates belong to the species *C. albicans*. Nonetheless, the natural diversity within this species, as well as the possibility of other species present in the oral cavity, highlight the need for a more comprehensive approach with larger samples to validate and extend our conclusions. Our findings align with those of several other studies, including the work by Mohammed et al. [13] who reported a prevalence of 47%, and Mohamadi et al. [30] who identified *C. albicans* at a rate of 64.4%.

In our investigation, we conducted in-vitro susceptibility testing of isolated strains to two antifungal agents. Fluconazole showed higher efficacy, with a susceptibility rate of 93.3%, followed by amphotericin B at 79.2%. Comparatively, the study by Tulasidas et al. [31] found that among species isolated from patients with vulvovaginal candidiasis, 79.1% showed susceptibility to fluconazole. Similarly, ElFeky et al. [18], observed a susceptibility rate of 77.8% for strains to this antifungal, while 98.4% of isolates were susceptible to amphotericin B. Furthermore, in the investigation by Marak &

Dhanashree [9], all tested *Candida* isolates exhibited susceptibility to amphotericin B.

The biofilm-forming ability of clinical *Candida* strains was investigated in this study. Various methods have been employed by researchers to investigate in vitro biofilm formation, with crystal violet standing out as one of the most common techniques for determining the total biomass of the biofilm. However, due to the non-specific character of this method, a supplementary approach based on the measure of metabolic activity should be explored. Many researchers have used reducing tetrazolium salts, to assess the biofilm-forming capacity in bacteria[21,32,33] and in fungal species [3,34,35]. In This investigation, quantification of biofilm using crystal violet staining revealed that the total biofilm biomass of *C. albicans* was comparatively low relative to *Candida* non *albicans* species. These findings are consistent with those reported by Muadcheingka & Tantivitayakul [3]. Conversely, Ferreira et al. [36] found no difference in biofilm production between the two groups. Among non-*albicans Candida* strains, *C. glabrata* exhibited the highest biofilm-producing capacity, with 33% of strains producing intense biofilm. This result contrasts with those of Tulasidas et al. [31] and Sahal & Bilkay [7], who reported significant biofilm formation in *C. tropicalis*, identified as the most intense biofilm-forming species. In another study conducted by Pathak et al. [37] and Silva et al. [38], it was reported that *C. glabrata* exhibits a strong ability to form biofilms on acrylic surfaces and silicone, respectively. These findings support the theory of several researchers that biofilm production does not depend on hyphal morphology, as *C. glabrata* is exclusively composed of blastospores and lacks hyphae [39].

In this study, TTC salt reduction was utilized for the measurement of metabolic activity. A correlation was observed between this method and crystal violet staining, indicating higher metabolic activity in non-*albicans Candida* species. Approximately 27% of *C. glabrata* strains exhibited intense metabolic activity, followed by *C. albicans* at 10%. Consistently, Sánchez-Vargas et al. [40] and Marcos-Zambrano et al. [34] similarly identified *C. glabrata* as having the highest metabolic activity. Conversely, Tulasidas et al. [31] observed higher metabolic activity in *C. tropicalis*. An intriguing observation pertained to *C. tropicalis*, which exhibited a significant biofilm biomass but relatively low metabolic activity. Similarly, Marcos-Zambrano et al. [34] reported a comparable finding, suggesting that this might be explained by the biofilm of this species' dense extracellular matrix, which might obstruct the flow of nutrients and oxygen. Our results demonstrated that this approach was suitable for detecting biofilms of *Candida* species, enabling visual detection through the formation of a red formazan precipitate whose intensity is correlated with the concentration of viable fungi [32]. Several studies, notably those by Sabaeifard et al. [21] and Attaran & Falsafi [41], have shown the effectiveness of TTC in evaluating biofilm formation in *Pseudomonas aeruginosa* and *Helicobacter pylori*, respectively. However, a study conducted by Brown et al. [42] demonstrated the inefficacy of TTC staining on *Campylobacter jejuni*.

Metabolic activity emerges as a key indicator of pathogenicity, given its frequent association with the expression of virulence factors and resistance to antifungal agents[36]. Tumbarello et al. [43] highlighted the substantial clinical impact of biofilm production, revealing higher mortality rates among patients infected by biofilm-forming strains compared to those infected by non-biofilm-forming strains, in addition to the greater time and cost of therapy. This finding aligns with the results of our investigation, where the majority of antifungal-resistant strains were found to be biofilm-forming. Similarly, Tulasidas et al. [31] noted a greater degree of resistance to fluconazole among biofilm-producing strains compared to non-producers.

Conclusion

This study revealed that *C. albicans* was the most frequently encountered species, followed by *C. glabrata*. Fluconazole exhibited superior efficacy compared to amphotericin B in terms of antifungal susceptibility. Moreover, our assessment of the biofilm-forming ability of clinical *Candida* strains, using CV staining and the TTC salt reduction test, showed significant variations, with *C. glabrata* displaying the highest capacity of biofilm production. Additionally, we noted a correlation between metabolic activity and

biofilm biomass across the strains. These findings demonstrate the clinical significance of biofilms in *Candida* infections and emphasize the imperative for further investigation to advance therapeutic approaches and detection methodologies, particularly for strains capable of generating biofilms.

Ethical Approval

For this study, an ethics committee's approval was not necessary since a hospital provided the clinical samples. In addition, this study was validated by the scientific committee of the Faculty of Life and Nature Sciences, Mustapha Stambouli University, Mascara, Algeria.

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نشرة العلوم الصيدليـــة
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القابلية المضادة للفطريات والتحليل المقارن لإنتاج الأغشية الحيوية والنشاط الأيضي في الكانديدا المعزولة من العينات السريرية

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تعرف Candida بأنها خميرة متعايشة في قنوات الفم والمهبل ويمكن أن تسبب مجموعة من الأمراض الانتهازية. قدرتها على تكوين البيوفيلم هي ما يكمن وراء العدوى ومن بين المسبب الرئيسي لها. هذه الدراسة تهدف إلى استكشاف القدرة على تكوين البيوفيلم والحساسية للأدوية المضادة للفطريات لعزلاتCandida . تم جمع العينات من المواقع المهبلية والفموية وتم تحديدها باستخدام اختبارات النمط الظاهري مثل CHROMagar، وcarbohydrate assimilation. تم تقدير الكتلة الحيوية الكلية للبيوفيلم والنشاط الأيضي باستخدام طريقة التلوين بالcrystal violet واختزال ملح tetrazolium، على التوالي. من بين ١٢٠ نوعًا من Candida المعزولة، تتصدر سلالة كان C. albicans (85%)، تليها بنسبة أقل C. glabrata (12.5%)، C. dubliniensis (1.66%)، و. C. tropicalis (0.83%) أظهرت غالبية العزلات حساسية عالية تجاه fluconazole (93.3%)، يليه amphotericin Bب (٧٩.٢%). لوحظ وجود ترابط كبير بين الكتلة الحيوية للبيوفيلم والنشاط الأيضي، حيث أظهرت سلالات non-albicans Candidaأعلى تكوين للأغشية الحيوية، تليها سلالات Candida albicans. تؤكد هذه النتائج أهمية مراعاة هذه العوامل عند التعامل مع عدوى .Candida