Sana'a University Journal of Medical and Health Sciences https://journals.su.edu.ye/index.php/jchm
Vol. 18 | No. 2 | Page 29 – 38 | 2024 | ISSN: 2958-7476
Doi: https://doi.org/10.59628/jchm.v18i2.971

In vitro sensitivity of *Candida albicans* isolates to antifungal agents and the effect of biofilms on the drug resistance rate

Ebtihal Mohamed Madar [1,3] , Azhar Mohammed Al-Ankoshy[2]
Hassan Abdulwahab Al-Shamahy [1,3,*] , Al-Kasem Mohammed A Abbas [4]

[1.] Department of Basic Sciences, Faculty of Dentistry Sana'a University, Sana'a, Yemen.
[2.] Jabir Ibn Hayyan Medical University, Faculty of Medicine, Department of Physiology, An-Najaf, Iraq.
[3.] Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen.
[4.] Department of Oral and Maxillo-Facial Surgery, Faculty of Dentistry, Sana'a University, Sana'a, Yemen.

***Corresponding author** *shmahe@yemen.net.ye*

ARTICLE INFO
Article history:
Received: Apr 23, 2024
Accepted: Jun 27, 2024
Published: Jun, 2024

KEYWORDS
1. Anti-fungal resistance
2. biofilm formation
3. Candida albicans
4. oral cavity
5. bucall mucosa

ABSTRACT

Background and objectives: The pathogenic species that is most frequently isolated from fungus infections is *Candida albicans*. The host's immune system, the severity of the infection, and the antifungal medication of choice all influence the management of these infections. Despite the advancement of novel antifungal medications, epidemiological investigations have demonstrated that resistance to antifungal agents in *C. albicans* strains is becoming a significant issue. The aims of this study were to (I) determine the prevalence of antifungal resistance in 53 oral *Candida albicans* isolates and (II) investigate the effect of biofilm production capacity on antifungal resistance rates.

Methods: Fifty-three isolates of *Candida albicans* were evaluated for biofilm production using the Tissue Culture Plate Method (TCPM). The antifungal susceptibility pattern of these isolates for amphotericin B, caspofungin, anidulafungin, voriconazole, itraconazole, ketoconazole, and posaconazole was determined using the E-test.

Results: The present study showed strong, moderate, weak, and negative biofilm production in 18.9%, 34.0%, 26.4%, and 20.8% of the *C. albicans* isolates, respectively. Amphotericin B, anidulafungin, and caspofungin effectively inhibited all of the tested isolates. The resistance rates of *C. albicans* isolates to fluconazole, itraconazole, ketoconazole, voriconazole, and posaconazole were 35.8%, 20.7%, 37.7%, 13.2%, and 20.7%, respectively. Strong and moderate biofilm-producing isolates displayed higher resistance rates compared to weak and negative biofilm producers. The majority of antifungal treatments showed a significant difference ($p < 0.05$)".

Conclusion: Our results suggest that the study area has a higher prevalence of azole-resistant *Candida albicans*. Thus, there is an urgent need to develop a plan to decrease the overuse and unneeded adverse effects of antifungal medications. Testing for antifungal susceptibility and fungus culture will be helpful in monitoring resistance and providing therapy for patients. It was observed that the development of *Candida albicans* biofilms was correlated with the drug resistance component of oral *Candida albicans* isolates.

CONTENTS

1. Introduction:

Fungal infections have increased over the past few decades due to the extensive use of corticosteroids, immunosuppressive drugs, antineoplastic medicines, and broad-spectrum antibiotics [1-3]. The pathogenic species that is most frequently isolated from these fungal infections is *Candida albicans* [4]. The host's immune system, the severity of the infection, and the antifungal medication of choice all influence how these infections are treated [5]. Novel antifungal medications have been created recently and put into clinical practice to treat fungal infections. Despite the advancement of novel antifungal medications, epidemiological investigations have demonstrated that treatment resistance in *C. albicans* strains is becoming a significant issue [6, 7]. The pathophysiology of candidiasis depends on the development of virulence factors such as adhesions, germ tube formation, phenotypic switching, biofilm formation, and the production of hydrolytic enzymes [8–10]. *Candida albicans*-related disorders are mostly caused by biofilm development. Biofilms are made up of microorganisms that grow complex three-dimensional structures on both biotic and abiotic surfaces while embedded in extracellular matrix (ECM) [11]. Biofilms have the ability to form on mucosal surfaces as well as the plastic surfaces of indwelling devices [12–14].

The most used class of antifungal medications is triazoles [3]. However, azole resistance in *Candida* species has emerged as a result of increased usage of triazoles in both empiric and preventive therapy [6]. Fluconazole resistance rates in *C. albicans* isolates have been found to be higher in numerous investigations [15, 16]. The more recent triazoles, posaconazole and voriconazole, exhibit broad-spectrum efficacy against molds and yeasts, including fluconazole-resistant *Candida* spp. [17,18]. Cross-resistance has been documented despite the fact that voriconazole and posaconazole are effective against fluconazole-resistant *Candida* spp. [18,19].

Given the frequent reports of treatment failure and rising antifungal resistance rates in *C. albicans* strains, in vitro antifungal susceptibility testing is a crucial technique for choosing an appropriate antifungal medication [15–17, 20–21]. Characterizing the alterations in *C. albicans* strains' antifungal sensitivity patterns is another benefit of antifungal susceptibility testing. For assessing the in vitro susceptibilities of *Candida* species to the azoles, amphotericin B, and caspofungin, the agar-based Etest is a valuable technique [22–25].

The current study aims are to isolate *Candida albicans* from bucall mucausa of denture patients, OFA patients, and normal healthy individuals, detect biofilm formation, and investigate their antifungal susceptibility pattern and its association with biofilm development. This is because there are very few studies on biofilm formation and drug resistance reported from Yemen.

2. Materials and methods

The isolated *Candida albicans* was then phenotypically identified using accepted methods in compliance with the Clinical and Laboratory Standards Institute's 2015 recommendations (CLSI) [26].

Biofilm production detection

Biofilm was identified using the tissue culture/microtiter plate technique (TCA) [27, 28]. Yeast isolates on fresh agar plates were covered with two milliliters of Brain Heart

Infusion (BHI) broth, and the plates were then incubated at 37°C for the entire day. Each microtitration plate received 200 µl of the sample that had been diluted 1:40 times with fresh medium (BHI broth supplemented with 1% glucose). The plates were then incubated for a further 24 hours at 37°C. Free floating sessile *Candida albicans* was removed by repeatedly rinsing it with phosphate buffered saline (pH 7.2) after gently tapping the contents. The yeast was maintained with 2% sodium acetate after attaching to the surface and creating biofilms, and it was then colored with 0.1% w/v crystal violet for ten to fifteen minutes. The plate was allowed to dry after the removal of the unbound crystal violet solution using three different PBS washes. After releasing the dye in each well with 200 l of 95% ethanol, an optical density (OD) measurement was made at 630 nm. Each test strain's OD values as well as those of the negative control were computed, and the OD cutoff values (ODc) were evaluated [28, 29].

Antifungal sensitivity testing (Epsilometer test)

Amphotericin B, voriconazole, caspofungin, fluconazole, ketoconazole, itraconazole, posaconazole, and anidulafungin were used in antifungal susceptibility studies.

The test was carried out in accordance with the manufacturer's instructions. 1.5% agar and 2% glucose were added to RPMI-1640 medium (Sigma, USA) before the agar plates were created. Furthermore, the solution was buffered to a pH of 7.0 using 0.165 mol L-1 MOPS (3-[N-morpholino] propanesulfonic acid) (Sigma, USA). Yeast colonies were suspended in saline, and the turbidity of the final inoculum was adjusted to 0.5 McFarland. The agar plates were inoculated by dipping a sterile swab into the suspension and swabbing the surface three times. After the plates were allowed to dry in a safety cabinet for fifteen minutes, test strips were applied to the agar surface using sterile forceps. For 24 to 48 hours, the plates were incubated at 35°C. Species-specific breakpoints from the Clinical and Laboratory Standards Institute's (CLSI) M27-S4 document were used to determine an isolate's sensitivity to itraconazole, voriconazole, fluconazole, and caspofungin [26].

Statistical Analysis: Version 7 of Epi-Info Statistics was used to analyze the data. To take into consideration the level of antibiotic resistance of 148 *Candida albicans* with different levels of biofilm formation, a statistical analysis was carried out. For each tested antifungal with a given quantity of biofilm development, the diffrance, 95% confidence interval, and p-value of the antifungal resistance were determined.

3. Results

The 310 participants in the study were divided into 104 groups—104 with dentures, 104 with orthodontic abaratus, and 102 controls without dental prostheses—with a mean ± SD of age equal to 37.01 ± 20.9 years old. Of these, 41.9% were male and 58.1 were female. The age group of 21–30 years old comprised the majority of participants (25.8%), followed by ≥51 years old (23.9%) and 31–40 years old (22.3%). 34.8% (108/310) of the samples had *Candida* colonization (Table 1).

Table 1: General characteristics of participate in the study

Characters	N (%)
Sex	
Male	130 (41.9)
Female	180 (58.1)
Ages (years)	
<21 years	50 (16.1)
21-30	80 (25.8)
31-40	69 (22.3)
41-50	40 (12.9)
≥51	74 (23.9)
Mean age	37.01Years
SD	20.9 Years
Mode	23 Years
Median	26 Years
Min-Max	9- 90 Years
Type of patients	
Denture	104 (33.5)

orthodentic	104 (33.5)
Normal	102 (32.9)
Total	310 (100)

The final statistical analysis included 310 qualified research participants in total. 34.8% of the 108 individuals with OCC had a prevalence rate. 108 OCC patients had 148 oral *Candida* spp. identified. *C. albicans* (49.1%) was the most often isolated species throughout our study, followed by *C. glabrata* (35.2%) and *C. dubliniensis* (13%), as shown by the species distribution in Table 2.

Table 2: Distribution of *Candida* strains isolated from denture, FOA and normal teeth individuals

Species	**n (%)**
Candida albicans	53 (49.1)
Candida glabrata	38 (35.2)
Candida dubliniensis	14 (13)
Candida tropicalis	15 (13.9)
Candida famata	8 (7.4)
Candida kefyr	8 (7.4)
Candida krusei	4 (3.7)
Candida parapsilosis	3 (2.8)
Candida africana	3 (2.8)
Candida stellatoidea	2 (1.9)
Single growth *candida* isolates	68/148 (45.9)
Mixed growth *candida* isolates	80 /148 (54.1)
Total *candida* isolates	148
Mono-infection cases	64/310 (20.6)
Co-infection cases	44/310 (14.2)
Positive candidaiasis cases	108/310 (34.8)

For the first time, *Candida kefyr*, *Candida krusei*, *Candida famata*, *Candida africana*, and *Candida stellatoidea* were isolated from the oral cavities of Yemeni dental patients. Moreover, mixed cultures of two to three species of *Candida* were found in 44 cases (14.2%) out of 310 people. The presence of non-albicans species was most frequently associated with co-infection with *Candida albicans* and/or *Candida glabrata*. Further details on co-infection of *Candida* species are included in Table 2. Table 3 gives the interpretation of biofilm development by the tested *Candida albicans* based on the average biofilm formation with an OD value obtained from the tissue culture plate method. Twenty.8% of the tested candida albicans showed a negative ability to build biofilms (OD < 0.17), and 26.4% showed a weak ability (OD = 0.17–0.34). 34% of the artificially isolated *Candida albicans* exhibited moderate positivity (OD = 0.35-0.68), while only 18.9% of the studied *Candida albicans* showed significant positive for biofilm production (OD > 0.68). The relationship between *Candida* biofilm development and antifungal resistance in isolates from patient buccal mucosa is displayed in Table 4. For example, the difference in fluconazole resistance was 45.1%, which indicates that biofilm-producing bacteria (moderate/strong) have a 45.1% resistance to Fluconazole compared to negative/weak strains. This result is highly statistically significant (p=0.0007), and the rate varies from 19.6 to 63.2%. In summary, compared to negative/weak biofilm-producing strains, moderate/strong biofilm-producing strains of *Candidaalbicans* exhibited a higher risk of drug resistance to the isoniconazole, ketoconazole, voriconazole, and posaconazole under investigation. Anidulafungin, capsofungin, and amphotericin B were all effective against *Candida albicans*. Isolates with minimum inhibitory concentrations (MICs-90%) of less than 0.064 µg/mL for amphotericin B, ≤256 µg/mL for ketoconazole, and >32 µg/mL for posaconazole were considered sensitive.It was shown that isolates with MICs for ketoconazole ranging from 0.002 µg/mL to 0.5 µg/mL were dose-dependently sensitive. It was shown that isolates with posaconazole MICs ≤ 0.25–0.5 µg/mL showed intermediate resistance. Reactions were considered resistant if their minimal inhibitory concentrations (MICs) for amphotericin B, ketoconazole, or posaconazole were at least 2 µg/mL, 1 µg/mL, or 1 µg/mL, respectively. The resistant rates for fluconazole, Itraconazole, ketoconazole, voriconazole, and posaconazole were 35.8%, 20.7%, 37.7%, 13.2%, and 20.7% respectively. All *Candida albicans* were sensitive to amphotericin B, anidulafungin, and capsofungin.

Table 3: Biofilm production by *candida albicans* isolates

OD value	N (%)
<0.17 Negative	11 (20.8)
0.17-0.34 Weak positive	14 (26.4)
0.35-0.68 Moderate positive	18 (34)
>0.68 Strong positive	10 (18.9)
Total	53 (100)

Table 4: Association of biofilm formation and Antifungal resistant of *Candida albicans* isolated

	Total n=53	Biofilm Negative/weak N=25	Biofilm Moderate/strong N=28	DF% (95% CI)	p value
Antifungal Agents	**Resistance N (%)**	**Resistance N (%)**	**Resistance N (%)**		
Fluconazole	19 (35.8)	3 (12)	16 (57.1)	45.1 (19.6-63.2)	0.0007
Itraconazole	11 (20.7)	3 (12)	8 (28.6)	16.6 (-5.7-36.7)	0.1
Ketoconazole	20 (37.7)	5 (20)	15 (53.6)	33.6 (7.4-53.8)	0.01
Voriconazole	7 (13.2)	2 (8)	5(17.9)	9.9 (-9.7-28.5)	0.29
Posaconazole	11 (20.7)	2 (8)	9 (32.1)	24.1 (1.9-43.5)	0.03
Amphotericin B	0 (0.0)	0 (0.0)	0 (0.0)	-	-
Anidulafungin	0 (0.0)	0 (0.0)	0 (0.0)	-	-
Caspofungin	0 (0.0)	0 (0.0)	0 (0.0)	-	-

DF=difference (%)

Table 5. Antifungal sensitivity testing of *Candida albicans*

Antifungal Agents	MIC, µg/mL			Resistant
	Range	50%	90%	
Fluconazole	0.064 - 256	1	> 256	19 (35.8)
Itraconazole	0.004 - 32	0.016	> 32	11 (20.7)
Ketoconazole	0.002 - 32	0.016	> 32	20 (37.7)
Voriconazole	0.002 - 32	0.012	> 32	7 (13.2)
Posaconazole	0.004 - 32	0.047	> 32	11 (20.7)
Amphotericin B	0.003 - 0.25	0.016	0.064	0 (0.0)
Anidulafungin	< 0.002 - 0.006	< 0.002	0.002	0 (0.0)
Caspofungin	0.012-0.5	0.19	0.38	0 (0.0)

4. Discussion

Biofilms are complex, multicellular, and mutually dependent communities of microorganisms that are adhered to surfaces and covered in an exopolysaccharide matrix. They're present on a variety of surfaces, including medical equipment's [8, 9]. The ability of *Candida* species to form biofilms, which is essential for virulence during candidiasis, is associated with their pathogenicity [33]. The current study found that twenty-eight (52.8%) of the *Candida albicans* generated biofilms, which is regarded as moderate or strong. This result is nearly the same as that reported by Kumar *et al*. [34]. The research participants' increased incidence of *Candida albicans* colonization and biofilm development in the oral mucosa may cause oral infections or extend to the digestive and respiratory systems. Douglas study supports this notion by demonstrating that biofilms, including bacterial and fungal biofilms, are responsible for over 80% of all microbial illnesses [35]. Because of structural and physiological features, the biofilms are innately resistant to antimicrobial

therapy as well as the host's immune system. Biofilms cause a wide range of diseases, including superficial mucosal infections and serious, widespread bloodstream infections. Biofilms that form on mucosal surfaces or on implanted medical devices, including dentures and FOA, are the most frequent source of these infections [8, 9].

In comparison to negative/weak biofilm-producing strains, moderate/strong biofilm-producing strains of *Candida albicans* showed a higher rate of medication resistance to the tested imitraconazole, ketoconazole, voriconazole, and posaconazole. This result can be explained by the fact that changes in metabolic states and constitutive activation of drug pumps cause cells in biofilms to become effectively resistant to medicines [36]. Because biofilms are hypothesized to offer fungus with physical protection from drugs, *Candida albicans* biofilms are also resistant to traditional antifungal medications. Four stages comprise the development of albicans biofilm in vitro:[35–39] Round yeast cells attach to surfaces and begin to colonize them; (2) yeast cells grow and proliferate, forming a basal layer of anchoring cells; (3) yeast cells grow into long cylindrical cells called hyphae and pseudohyphae, which grow in tandem with the production of extracellular matrix; and (4) yeast cells disperse from the biofilm to find new sites to colonize.

Monitoring antifungal resistance in *Candida albicans* is crucial because it can reveal newly developing risks posed by resistant forms of the infection, which can help with empirical treatment. All 53 of the *Candida albicans* isolates that we analyzed turned out to be amphotericin B sensitive, which is in line with the results reported by Arora *et al*. [40]. Additionally, similar outcomes have been noted in earlier research conducted in European nations [41–44]. Badiee and Alborzi [19] have shown that in Southern Iran, the amphotericin B resistance rate of *C. albicans* isolates was 7%. As per our findings, 35.8% of the *C. albicans* strains isolated from candiduria had fluconazole resistance. Similarly, studies by Zarei Mahmoudabadi *et al*. [45] demonstrated that 55.2% of these strains were resistant to the drug. The same authors also found that *C. albicans* had a 59.2% fluconazole resistance rate in another investigation [45]. Previous studies have revealed minimal resistance rates for fluconazole [42, 44, 46-51], which is in contrast to our findings. Additionally, our sample's resistance rates to voriconazole, itraconazole, and fluconazole were higher than those seen in a prior Turkish investigation [41]. Diverse breakpoint values, azole exposure in the past, and variations in the patient population could all contribute to variations in these resistance rates. It is crucial to highlight that in order to assess C. albicans strain susceptibility to fluconazole, itraconazole, and voriconazole, CLSI recently defined new species-specific MIC breakpoints. The impact of new MIC breakpoints on azole and echinocandin resistance patterns in *Candida* species was assessed by Fothergill et al. [52]. It was discovered that isolates of *Candida albicans* had resistance rates higher than those previously determined [30] when analyzed using the new CLSI criteria.

Based on our findings, the current study's resistance rates to fluconazole, itraconazole, ketoconazole, posaconazole, and voriconazole were 35.8%, 20.7%, 37.7%, 20.7%, and 13.2%, in that order. Yenisehirli *et al.* reported that the frequencies of fluconazole and voriconazole resistance in *C. albicans* were 34% and 14%, respectively, in comparable ranges [30]. Also, according to research published by Jayalaksmi *et al. [53*], 105 *Candida albicans* that were collected from diverse clinical specimens exhibited a 34.3% fluconazole resistance rate. A study by Pelletier *et al. [54*] found that 42 out of 295 (14.2%) *Candida albicans* isolates had reduced fluconazole susceptibility. Our rates of resistance to voriconazole and fluconazole are in line with those observed in earlier studies. It's possible that the study participants' lengthy and

heavy use of voriconazole and fluconazole reduced their susceptibility to those antifungals. Additionally, our 34.3% fluconazole resistance rate is lower than the 55.2% of *C. albicans* strains that were isolated from candiduria that had fluconazole resistance, according to research by Zarei Mahmoudabadi *et al. [55]*. In another study, the same authors discovered that *C. albicans* had a fluconazole resistance rate of 59.2% [47]. Additionally, the percentage of fluconazole-resistant *Candida albicans* found in this inquiry was 35.8%, which is greater than the percentage found in investigations by Mohamed and Al-Ahmadey [56] and Nemati *et al.* [57], where the range of fluconazole resistance in *Candida albicans* was 0% to 15% [56,57]. Furthermore, studies on the efficacy of fluconazole against *Candida albicans* have demonstrated that 75% of strains tested were susceptible. Compared to the 95%, 87.5%, and 89.5% rates that Badiee and Alborzi [19], Citak *et al.* [58], and Mohamed and Al-Ahmadey [56] previously published, this sensitivity rate is not as comparable. Furthermore, consistent with the results of Ng *et al.* [59], who reported on the amphotericin B and ketoconazole susceptibility of all yeast isolates. The percentage of *Candida albicans* that is resistant to antifungal agents may rise as a result of short courses of antifungal therapy, long-term use of suppressive azoles, and widespread usage of antifungal medications [59].

5. Conclusion

According to our research, the formation of biofilms may contribute to the emergence of drug resistance. A major problem for the medical community is that most yeast, including *Candida albicans*, are found in biofilm form. Due to its innate immune response and anti-fungal properties, *Candida albicans* is extremely difficult to treat since it depends on biofilms to thrive. The creation of anti-biofilm medications and mouthwashes that stop the formation of biofilms depends on an understanding of the mechanisms behind the production and regulation of oral candida biofilms.

Ethical Consideration: The Faculty of Medicine and Health Sciences at Sana'a University received ethical authority for the Contract No. 217 project on August 21, 2022, from the Medical Ethics and Research Committee. The review committee's set of ethical guidelines was regularly adhered to. The chosen individuals provided their informed and signed consent.

Acknowledgments

The authors would like to thank Yemen and the Faculty of Dentistry at Sana'a University for their kind cooperation.

A dispute of interest: There are no conflicts of interest in regard to this project.

Author's contributions

Ibtihal M Madar, the study's second author, conducted the fieldwork for her PhD at Sana'a University's Faculty of Medicine and Health Sciences' Department of Medical Microbiology. Additional authors contributed to the data analysis, manuscript drafting and review, and research approval.

6. References

[1] Ortega M, Marco F, Soriano A, Almela M, Martinez JA, Lopez J, et al. Candida species bloodstream infection: epidemiology and outcome in a single institution from 1991 to 2008. *J Hosp Infect.* 2011;**77**(2):157–61.

[2] Holzheimer RG, Dralle H. Management of mycoses in surgical patients -- review of the literature. *Eur J Med Res.* 2002;**7**(5):200–26.

[3] Lass-Florl C. The changing face of epidemiology of invasive fungal disease in Europe. *Mycoses.* 2009;**52**(3):197–205.

[4] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev.* 2007;**20**(1):133–63.

[5] White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev.* 1998;**11**(2):382–402.

[6] Masia Canuto M, Gutierrez Rodero F. Antifungal drug resistance to azoles and polyenes. *Lancet Infect Dis.* 2002;**2**(9):550–63.

[7] Mukherjee PK, Sheehan D, Puzniak L, Schlamm H, Ghannoum MA. Echinocandins: are they all the same? *J Chemother.* 2011;**23**(6):319–25.

[8] Alhasani A H, Al-Akwa AAY, Al-Shamahy HA, Al-deen HS, Al-labani AM. "Biofilm formation and antifungal susceptibility of candida isolates from oral cavity after the introduction of fixed orthodontic appliances". Universal J Pharm Res 2020; 5(4): 1-6. https://doi.org/10.22270/ujpr.v5i4.435.

[9] Alhasani AH, Ishag RA, Yahya Al-Akwa AY, Al Shamahy HA, Al-labani MA. Association between the Streptococcus mutans biofilm formation and dental caries experience and antibiotics resistance in adult females. Universal Journal of Pharmaceutical Research 2020; 5(6):18-23. https://doi.org/10.22270/ujpr.v5i6.507

[10] Deorukhkar SC, Saini S, and Mathew S, "Virulence factors contributing to pathogenicity of Candida tropicalis and its antifungal susceptibility profile," International Journal of Microbiology 2014; 2014, Article ID 456878, 6 pages, 2014.

[11] Harriott MM, Lilly EA, Rodriguez TE, Fidel PL, Noverr MC, "Candida albicans forms biofilms on the vaginal mucosa," Microbiology, vol. 156, no. 12, pp. 3635–3644, 2010.

[12] Saleh AAM, Al-Shamahy HA, Al-Hrazi RM, B. et al. . "Biofilm formation and antibiotic susceptibility of uropathogens in patients with catheter associated urinary tract infections in ibb city -yemen". Universal J Pharm Res 2020; 4(6): 1-8, http://doi.org/10.22270/ujpr.v4i6.329.

[13] Al-Kebsi AM, Othman MO , Al-Shamahy HA, et al. Oral C. albicans colonization and non-candida albicans candida colonization among university students, Yemen. Universal J Pharm Res 2017; 2(5):5-11. http://doi.org/10.22270/ujpr.v2i5.R2

[14] Al-Sanabani NF, Al-Kebsi AM, Al-Shamahy HA, Abbas AKM. Etiology and risk factors of stomatitis among Yemeni denture wearers. Universal J Pharm Res 2018; 3 (1): 69–73. https://doi.org/10.22270/ujpr.v3i1.R9

[15] Grant SM, Clissold SP. Fluconazole. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mycoses. *Drugs.* 1990;**39**(6):877–916.

[16] Antoniadou A, Torres HA, Lewis RE, Thornby J, Bodey GP, Tarrand JP, et al. Candidemia in a tertiary care cancer center: in vitro susceptibility and its association with outcome of initial antifungal therapy. *Medicine (Baltimore).* 2003;**82**(5):309–21.

[17] Nguyen MH, Yu CY. Voriconazole against fluconazole-susceptible and resistant candida isolates: in-vitro efficacy compared with that of itraconazole and ketoconazole. *J Antimicrob Chemother.* 1998;**42**(2):253–6.

[18] Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother.* 2006;**50**(6):2009–15.

[19] Badiee P, Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: A five year study. *Iran J Microbiol.* 2011;**3**(4):183–8.

[20] Baixench MT, Aoun N, Desnos-Ollivier M, Garcia-Hermoso D, Bretagne S, Ramires S, et al. Acquired resistance to echinocandins in Candida albicans: case report and review. *J Antimicrob Chemother.* 2007;**59**(6):1076–83.

[21] Kontoyiannis DP, Lewis RE. Antifungal drug resistance of pathogenic fungi. *Lancet.* 2002;**359**(9312):1135–44.

[22] 22- Chryssanthou E, Cuenca-Estrella M. Comparison of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing proposed standard and the E-test with the NCCLS broth microdilution method for voriconazole and caspofungin susceptibility testing of yeast species. *J Clin Microbiol.* 2002;**40**(10):3841–4.

[23] Pfaller MA, Messer SA, Mills K, Bolmstrom A, Jones RN. Evaluation of Etest method for determining posaconazole MICs for 314 clinical isolates of Candida species. *J Clin Microbiol.* 2001;**39**(11):3952–4.

[24] Pfaller MA, Messer SA, Bolmstrom A. Evaluation of Etest for determining in vitro susceptibility of yeast isolates to amphotericin B. *Diagn Microbiol Infect Dis.* 1998;**32**(3):223–7.

[25] Pfaller MA, Messer SA, Mills K, Bolmstrom A, Jones RN. Evaluation of Etest method for determining caspofungin (MK-0991) susceptibilities of 726 clinical isolates of Candida species. *J Clin Microbiol.* 2001;**39**(12):4387–9.

[26] Clinical and Laboratory Standards Institute . Reference method for broth dilution antifungal susceptibility testing of yeasts; Fourth informational supplement. .Wayne: CLSI document M27-S4; 2012.

[27] Christensen GD, Simpson WA, Bisno AL, Beachley EH. Adherence of slim-producing strains of staphylococcus epidermidis to smooth surfaces. Infect Immunity 1982; 37(1):318–326. PMID:6179880

[28] Stepanovic S, Vukovic D, Hola V, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by Staphylococci. Acta Patho Micro 2007; 115(8):891–899. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x

[29] Diaz PI, Valm AM. Microbial Interactions in Oral Communities Mediate Emergent Biofilm Properties. J Dent Res. 2020 Jan;99(1):18-25. https://doi.org/10.1177/0022034519880157. Epub 2019 Oct 7. PMID: 31590609; PMCID: PMC6927214.

[30] Yenisehirli G, Bulut N, Yenisehirli A, Bulut Y. In Vitro Susceptibilities of Candida albicans Isolates to Antifungal Agents in Tokat, Turkey. Jundishapur J Microbiol. 2015 September; 8(9): e28057. https://doi.org/10.5812/jjm.28057

[31] Pfaller MA, Espinel-Ingroff A, Canton E, Castanheira M, Cuenca- Estrella M, Diekema DJ, et al. Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and Candida spp. as determined by CLSI broth microdilution. J Clin Microbiol. 2012;**50**(6):2040–6.

[32] Candido RC, Toloi MR, Franceschini SA, Garcia FR, Minto EC. In vitro activity of antimycotic agents determined by E-test method against vaginal Candida species. Mycopathologia. 1998;**144**(1):15–20.

[33] 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. J Glob Infect Dis. 2011 Jan;3(1):4-8. https://doi.org/10.4103/0974-777X.77288. PMID: 21572601; PMCID:

[34] Jabra-Rizk MA, Falkler WA, and Meiller TF, "Fungal biofilms and drug resistance," Emerging Infectious Diseases 204; 10(1):14–19.

[35] Douglas LJ. Candida biofilms and their role in infection. Trends Microbiol 2003; 11:30-6; PMID: 12526852; http://dx.doi.org/10.1016/S0966-842X(02)00002-1

[36] Emily P. Fox; Clarissa J. Nobile. A sticky situation, Transcription 2012; 3:6, 315-322. https://doi.org/10.4161/trns.22281

[37] Hawser SP, Douglas LJ. Biofilm formation by Candida species on the surface of catheter materials in vitro. Infect Immun 1994; 62:915-21. https://doi.org/10.1128/IAI.62.3.915-921.1994

[38] Baillie GS, Douglas LJ. Role of dimorphism in the development of Candida albicans biofilms. J Med Microbiol 1999; 48:671-9. https://doi.org/10.1099/00222615-48-7-671

[39] Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol 2001; 183:5385-94. https://doi.org/10.1128/jb.183.18.5385-5394.2001

[40] S. Arora, N. Dhuria, N. Jindal, and S. Galhotra, "International journal of research and development in pharmacy & life science speciation, biofilm formation and antifungal susceptibility of Candida isolates," International Journal of Research and Development in Pharmacy and Life Sciences 2017; 6(2517):2521.

[41] Bicmen C, Doluca M, Gulat S, Gunduz AT, Tuksavul F. Species level identification and antifungal susceptibility of yeasts isolated from various clinical specimens and evaluation of Integral System Yeasts Plus. *New Microbiol.* 2012;**35**(3):327–34.

[42] Fleck R, Dietz A, Hof H. In vitro susceptibility of Candida species to five antifungal agents in a German university hospital assessed by the reference broth microdilution method and Etest. *J Antimicrob Chemother.* 2007;**59**(4):767–71.

[43] Faria-Ramos I, Neves-Maia J, Ricardo E, Santos-Antunes J, Silva AT, Costa-de-Oliveira S, et al. Species distribution and in vitro antifungal susceptibility profiles of yeast isolates from invasive infections during a Portuguese multicenter survey. *Eur J Clin Microbiol Infect Dis.* 2014;**33**(12):2241–7.

[44] Bourgeois N, Dehandschoewercker L, Bertout S, Bousquet PJ, Rispail P, Lachaud L. Antifungal susceptibility of 205 Candida spp. isolated primarily during invasive Candidiasis and comparison of the Vitek 2 system with the CLSI broth microdilution and Etest methods. *J Clin Microbiol.* 2010;**48**(1):154–61.

[45] Zarei Mahmoudabadi A, Zarrin M, Beheshti Fard M. Antifungal susceptibility of Candida species isolated from candidura. *Jundishapur J Microbiol.* 2012;**6**(1):24–8.

[46] Zarei Mahmoudabadi A, Zarrin M, Kiasat N. Biofilm Formation and Susceptibility to Amphotericin B and Fluconazole in Candida albicans. *Jundishapur J Microbiol.* 2014;**7**(7):e17105.

[47] Santhanam J, Nazmiah N, Aziz MN. Species distribution and antifungal susceptibility patterns of Candida species: Is low susceptibility to itraconazole a trend in Malaysia? *Med J Malaysia.* 2013;**68**(4):343–7.

[48] Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ, et al. Use of fluconazole as a surrogate marker to predict susceptibility and resistance to voriconazole among 13,338 clinical isolates of Candida spp. Tested by clinical and laboratory standards institute-recommended broth microdilution methods. *J Clin Microbiol.* 2007;**45**(1):70–5.

[49] Santos ER, Dal Forno CF, Hernandez MG, Kubica TF, Venturini TP, Chassot F, et al. Susceptibility of Candida spp. isolated from blood cultures as evaluated using the M27-A3 and new M27-S4 approved breakpoints. *Rev Inst Med Trop Sao Paulo.* 2014;**56**(6):477–82.

[50] Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ. Selection of a surrogate agent (fluconazole or voriconazole) for initial susceptibility testing of posaconazole against Candida spp.: results from a global antifungal surveillance program. *J Clin Microbiol.* 2008;**46**(2):551–9.

[51] Shokohi T, Bandalizadeh Z, Hedayati MT, Mayahi S. In vitro antifungal susceptibility of Candida species isolated from orofaringeal lesions of patients with cancer to some antifungal agents. *Jundishapur J Microbiol.* 2011;**4**:19–26.

[52] Fothergill AW, Sutton DA, McCarthy DI, Wiederhold NP. Impact of new antifungal breakpoints on antifungal resistance in Candida species. *J Clin Microbiol.* 2014;**52**(3):994–7.

[53] Jayalakshmi L, Ratnakumari G, and Samson SH, "Isolation, speciation and antifungal susceptibility testing of Candida from clinical specimens at a tertiary care hospital," School Journal of Applied Medical Science, 2014; 2:3193–3198.

[54] Pelletier R, Loranger L, Marcotte H, and De Carolis E, "Voriconazole and fluconazole susceptibility of Candida isolates," Journal of Medical Microbiology 2002; 51(6):479–483.

[55] Zarei Mahmoudabadi A, Zarrin M, Beheshti Fard M. Antifungal susceptibility of Candida species isolated from candidura. Jundishapur J Microbiol. 2012;**6**(1):24–8.

[56] Ali MS, Zakey AAZ. Biofilm formation and antifungal susceptibility of Candida iisolates from various clinical specimens. British Microbiol Res J 2013; 3(4): 590-601. https://doi.org/10.9734/BMRJ/2013/5028

[57] Nemati SL, Shams-Ghhfarokhi M, Yadegari MH. Evaluation of disk diffusion and microdilution methods for Fluconazole susceptibility testing in one group of Candida spp. in Tehran. Daneshavar Med 2008; 15:51-58.

[58] Citak S, Ozcelik B, Cesur S and Abbasoglu U. In vitro susceptibility of Candida species isolated from blood culture to some antifungal agents. Jpn J Infect Dis 2005; 58(1):44-6. PMID: 15728993

[59] Ng KP, Saw TL, Na SL, Soo-Hoo TS. Systemic Candida infection of University Hospital 1997- 1999: the distribution of Candida biotypes and antifungal susceptibility patterns. Mycopathologia 2000; 149(3):141-146. https://doi.org/10.1023/a:1007283211220