Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2025;14(3):828-33

Investigation of biofilm production and hemolytic activity in various *Candida* isolates

Merih Simsek[1], Cengiz Demir[2]

[1]*Afyonkarahisar Health Sciences University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Afyonkarahisar, Türkiye*
[2]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Microbiology, Afyonkarahisar, Türkiye*

Received 02 April 2025; Accepted 22 April 2025
Available online 01 September 2025 with doi: 10.5455/medscience.2025.04.089

Abstract

In this study, it was aimed to investigate and compare biofilm production and hemolytic activity in various *Candida* isolates obtained from different clinical specimens. A total of 100 clinical isolates, 53 *C. albicans* and 47 non *albicans Candida* strains, were included in the study. The identification of strains was carried out using conventional methods and Vitek-2 system. Hemolytic activity was determined using 5% sheep blood SDA medium, and biofilm formation was determined using Christensen macro tube method. 53% of the isolates were *C. albicans*, 13% *C. famata*, 8% *C. kefyr*, 3% *C. parapsilosis*. Thus, 52% of the samples were determined as blood culture, 20% as wound, 10% as sputum. Moreover, 14% of 100 *Candida* strains isolated were isolated from Neonatal Intensive Care, 14% from General Surgery and 10% from Internal Intensive Care units. While 32% of 100 *Candida* strains were alpha hemolysis and 36% beta hemolysis was observed, 20% of these strains showed weak, 18% moderate, and 15% strong positive biofilm grades. Antifungal susceptibility testing demonstrated that *C. famata* exhibited the lowest sensitivity, particularly to fluconazole and flucytosine. The increase in the formation of strong positive biofilms suggests that increase the infectious properties and the antifungal resistance rate of *Candida*. It is of great importance to investigate the prevalence of biofilm formation and hemolysis production among *Candida* strains with multi-center studies and to follow them periodically, together with other virulence factors for the prevention and effective treatment of *Candida* infections.

Keywords: Biofilm, hemolytic activity, *Candida albicans*, non-*albicans Candida*

Introduction

Candida species are yeast species that are part of the normal human skin and intestinal flora. However, these microorganisms can become pathogenic when the immune system is suppressed and cause chronic mucocutaneous and invasive infections. These yeasts are microorganisms that can live in a solid formation called a biofilm and produce many different hydrolytic enzymes. Examples of enzymes it creates are protease, lipase, phospholipase, esterase and phosphatase enzymes. Hemolysis and biofilm formation have been reported among virulence factors [1,2]. *Candida* species can cause opportunistic infections in immunocompromised individuals. In particular, the forms of candidiasis caused by *Candida albicans* are quite numerous. *Candida* species frequently encountered in humans were determined as *C. albicans*, *C. tropicalis*, *C. parapsilosis*. The most important virulence factor of *Candida* species is biofilm production. The biofilm structure is a glycocalyx material and consists of 27% protein and 40% carbohydrates. However, the main component of a biofilm is water. Besides water and bacterial cells, the biofilm matrix is primarily a complex of exopolysaccharides. Biofilm matrix also contains proteins, DNA and products of bacterial degradation. Biofilm conditions are favorable for microorganisms.

A biofilm is a dynamic environment composed of micro-colonies of cells contained within the extracellular matrix. Biofilm is a feeding environment for microorganisms, but also acts as a defense mechanism against environmental stress [1]. Biofilms formed by *Candida* species consist of two layers. The lower layer is usually composed of yeasts firmly attached to the surface, while the upper layer is usually composed of hyphae. However, the final architecture of biofilms varies according to the substrate used for energy and growth conditions. Thanks

CITATION
Simsek M, Demir C. Investigation of biofilm production and hemolytic activity in various *Candida* isolates. Med Science. 2025;14(3):828-33.

Corresponding Author: Merih Simsek, Afyonkarahisar Health Sciences University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Afyonkarahisar, Türkiye
Email: smerih16@gmail.com

DOI: 10.5455/medscience.2025.04.089

to biofilms, *Candida* species gain resistance to antimicrobial agents, toxic substances and environmental threats such as oxidative stress [2]. In addition, it was determined that the biofilm caused a decrease in leukocyte and lymphocyte activity. It has been reported that biofilm production increases the risk of catheter infections caused by *Candida* species at high rates [3]. The use of medical devices such as joint prostheses and catheters is increasing day by day in the hospital environment. There is a close relationship between the frequency of use of these tools and the morbidity and mortality rates of fungal infections. Biofilms can lead to the formation of plaque on the teeth, causing tooth decay and paradontosis. However, it can also lead to the development of biofilm in medical devices that are in the high-risk group for sterilization, such as catheters or implants. Biofilms formed on the device cause encapsulation and aggregation of fungal cells. Therefore, they form phenotypically and morphologically different colonies that are highly resistant to antimicrobial agents compared to other *Candida* species [4-6]. Determining the biofilm prevalence rate among *Candida* species may contribute to the investigation of biofilm formation and its underlying biochemical mechanisms. This will help develop a more effective treatment strategy for biofilm infections. In this study, it was aimed to investigate biofilm production and hemolytic activity in *Candida* isolates isolated from various clinical samples.

Material and Methods

Microorganisms

A total of 100 clinical *Candida* isolates, comprising 53 *C. albicans* and 47 non-*albicans Candida* strains, were included in this study. These isolates were obtained from various clinical samples belonging to the culture collection collected between 2017 and 2019 at the Microbiology Laboratory of Afyonkarahisar Health Sciences University, Health Application and Research Center.

Identification of Microorganisms

Yeasts were identified by conventional methods such as Gram staining, culture cultivation on Sabouraud Dextrose Agar (SDA) supplemented with antibiotics, and the germ tube test. Identification and antifungal susceptibility tests of the strains grown in culture and determined to be yeast were performed using the Vitek-2 (bioMerieux, France) automated system, using Vitek-2 YST and Vitek-2 AST-YS07 cards, in accordance with the manufacturer's instructions. Vitek-2 automated system is a method that can identify microorganisms according to their biochemical properties and determine sensitivity.

Biofilm Formation

In the determination of biofilm formation, sterile V-bottom test tubes (15 cm) were used. In the study, fungus cultured in 10 ml of 8% glucose-containing liquid SDA medium were stained with 1% safranine after incubation at 37°C for 48 hours. The dye was washed 2 times with distilled water. Then, the presence of a visible thin film layer on the inner wall of the tubes was observed. Samples with a thin film layer were accepted as biofilm positive. Results, according to the thickness of the formed layer; negative (-), weak (+), moderate (++) or strong (+++). These evaluations were made by two independent researchers.

Hemolytic Activity

In order to determine hemolytic activities and hemolysis production, *Candida* strains were passaged in SDA medium after incubation at 37°C for 48 hours. Suspensions of McFarland 2-2.5 turbidity were prepared from the colonies, and 10 µl of 3% glucose and 5% sheep blood agar were spot seeded from these suspensions. Zones formed around point plantings in media kept in an environment of 5% CO_2 at 37°C for five days; beta hemolysis (transparent color), alpha hemolysis (green color) and gamma hemolysis (no hemolysis) were evaluated. Ethical approval was obtained for this study from the local non-interventional ethics committee. The principles in the Declaration of Helsinki were complied with.

Statistical Analysis

SPSS 20.0 statistical program was used for the analysis of the data. The difference between biofilm production and hemolysin production of the isolates was evaluated with the chi-square test and Fisher's exact test. Values with $p<0.05$ were considered significant. It was determined that 53 *C. albicans* and 47 non-*albicans* strains isolated from the patients included in this study produced biofilm factors. When these two groups were evaluated statistically, no significant difference was found in terms of biofilm factor production ($p>0.05$). When the production of hemolysin between *C. albicans* and non-*albicans* groups was evaluated statistically, the difference between them was found to be statistically significant ($p=0.033$).

Ethical Approval

Ethical approval was obtained for the study from the Afyonkarahisar Health Sciences Clinical Research Ethics Committee (decision no: 2019/200, and date: 14.06.2019). The principles in the Declaration of Helsinki were complied with.

Results

In this study, biofilm formation and hemolytic activity of *Candida* species (n=100) isolated from various clinical specimens were investigated. Of the patients with *Candida* species overgrowth, 57 (57%) were male and 43 (43%) were female. The mean age of the patients was determined as 45.47±21.23 (0-89 years). The clinical samples in which microorganisms reproduce most frequently were determined as blood culture, wound and sputum samples, respectively. The clinics where microorganisms were most frequently isolated were Neonatal Intensive Care and General Surgery clinics. The distribution of clinical samples and clinics from which *Candida* strains were isolated is detailed in Table 1. In this study, biofilm production was observed in 53% of 100 *Candida* strains, while biofilm production was not observed in 47%.

DOI: 10.5455/medscience.2025.04.089

Table 1. Distribution of *Candida* species according to clinical samples and clinics

Clinical samples	%
Blood culture	52
Tracheal aspirate	6
Urine	8
Wound	20
Sputum	10
Cerebrospinal fluid	4
Clinics	**%**
Anesthesia Intensive Care	6
Neurosurgery Intensive Care	7
Child Health and Diseases	5
Pediatric Hematology and Oncology	9
Internal Intensive Care	10
General Surgery	14
Chest Diseases	9
Medical Oncology Clinic	6
Newborn Intensive Care	14
Other	20

However, hemolytic activity was observed in 68% of these strains and no hemolytic activity was observed in 32% of them. Biofilm production rates and *Candida* rates showing hemolytic activity according to clinical samples are given in Table 2 in detail. While alpha hemolysis was observed in 32% of all *Candida* species and beta hemolysis was observed in 36%, 20% of these strains showed weak, 18% moderate and 15% strong biofilm. *Candida* species distribution according to the type of hemolysis and *Candida* species distribution according to the degree of biofilm layer are given in Tables 3 and 4 in detail. According to the antifungal susceptibility test results, it showed the lowest sensitivity against *C. famata*, fluconazole (8%) and flucytosine (23%). However, a large proportion of the tested microorganisms were found to be susceptible to antifungals. The distribution of *Candida* species and the antifungal susceptibility rates by species are given in Table 5.

Table 2. Rates of *Candida* showing biofilm production and hemolytic activity according to clinical samples

Clinical samples (n)	Biofilm production (%)	Hemolytic activity (%)
Blood culture (52)	38	54
Tracheal aspirate (6)	100	100
Urine (8)	100	88
Wound (20)	35	70
Sputum (10)	100	90
Cerebrospinal fluid (4)	50	75

Table 3. *Candida* species distribution by type of hemolysis

Candida species	Hemolytic activity (n/%)		
	Alpha hemolysis	Beta hemolysis	Gamma hemolysis
***C. albicans* (53)**	**15/28**	**26/49**	**12/23**
***C. parapsilosis* (3)**	-	-	3/100
***C. tropicalis* (2)**	-	-	2/100
***C. dubliniensis* (2)**	1/50	1/50	-
***C. glabrata* (3)**	-	-	3/100
***C. krusei* (2)**	-	1/50	1/50
***C. famata* (13)**	8/62	-	5/38
***C. kefyr* (8)**	1/13	7/87	-
***C. ciferrii* (2)**	-	-	2/100
***Candida spp.* (12)**	7/58	1/9	4/33
Non-*albicans* (47)	**17/36**	**10/21**	**20/43**

Table 4. *Candida* species distribution by degree of biofilm layer

Candida species	Biofilm production (n/%)			
	Negative (-)	Weak positive (+)	Medium positive (++)	Strong positive (+++)
***C. albicans* (53)**	**23/43**	**13/25**	**11/21**	**6/11**
***C. parapsilosis* (3)**	-	-	-	3/100
***C. tropicalis* (2)**	-	-	-	2/100
***C. dubliniensis* (2)**	2/100	-	-	-
***C. glabrata* (3)**	-	1/33	2/77	-
***C. krusei* (2)**	-	-	1/50	1/50
***C. famata* (13)**	6/47	3/23	2/15	2/15
***C. kefyr* (8)**	8/100	-	-	-
***C. ciferrii* (2)**	1/50	1/50	-	-
***Candida spp.* (12)**	7/58	2/17	2/17	1/8
Non-*albicans* (47)	**24/51**	**7/15**	**7/15**	**9/19**

DOI: 10.5455/medscience.2025.04.089

Table 5. *Candida* species distribution and distribution of antifungal susceptibility rates by species

Candida species	Amphotericin -B (n/%)	Caspofungin (n/%)	Fluconazole (n/%)	Flucytosine (n/%)	Micafungin (n/%)	Voriconazole (n/%)
***C. albicans* (53)**	53/100	53/100	49/92.5	53/100	53/100	48/90.6
***C. parapsilosis* (3)**	3/100	3/100	3/100	3/100	3/100	3/100
***C. tropicalis* (2)**	2/100	2/100	2/100	2/100	2/100	2/100
***C. dubliniensis* (2)**	2/100	2/100	2/100	2/100	2/100	2/100
***C. glabrata* (3)**	3/100	3/100	2/66.7	1/33.3	3/100	3/100
***C. krusei* (2)**	2/100	2/100	0 *	1/50	2/100	2/100
***C. famata* (13)**	13/100	13/100	1/7.7	3/23	13/100	6/46.1
***C. kefyr* (8)**	8/100	8/100	8/100	8/100	8/100	8/100
***C. ciferrii* (2)**	2/100	2/100	2/100	2/100	2/100	2/100
***Candida spp.* (12)**	9/75	11/91.7	4/33.3	4/33.3	11/91.9	6/50

*: Natural resistant

Discussion

Nowadays, due to the current increases in antifungal drug resistance of *Candida* species in immunocompromised patients, an increase in infections and thus treatment failures is observed. Although *C. albicans* is the most commonly isolated from such patients, it spreads rapidly in infections belonging to non-*albicans* species. The development of resistance during or after treatment is very worrying. Since long-term antifungal therapy may cause mutations in susceptibility genes to antifungals, the treatment of these infections has a challenging process [7,8].

Candida species have many virulence factors that play a role in the pathogenesis of the infections they cause. Hemolysis activity and biofilm formation are the most important virulence factors [9]. The hemolysin enzyme disrupts the iron metabolism in human erythrocytes and causes the cells to break down and hemoglobin to be thrown out. Thus, the ejected iron is used by *Candida*. Biofilm, on the other hand, participates in the teichoic acid structure and thus protects *Candida* from human immune system mechanisms [10-12].

The prevalence, isolation and virulence factors of *Candida* species have been investigated in many studies conducted in Turkey and around the world. In a study by Alpay et al., 64% of 53 *Candida* strains were *C. albicans*, 21% were *C. parapsilosis*, 8% were *C. kefyr*, and they were determined as the top three breeding species. Of the samples from which *Candida* species were isolated, 60% were urine, 15% were wounds, 9% were throat, and 8% were sputum [13]. According to the research results of Yıldırım et al., 70% of 132 *Candida* strains were *C. albicans*, 12% *C. parapsilosis*, 6% *C. tropicalis*, 3% *C. glabrata*, 3% *C. famata*, 2% *C. sake*, 2% *C. krusei* and 2% *C. kefyr*. Of the samples, 41% were urine, 26% blood, 8% sputum, 5% wound, 9% catheter, 4% vaginal, 3% aspirate material. The 19% of *Candida* strains were isolated from Internal Medicine clinics and 28% from Surgery clinics [14].

In this study, when the highly isolated species were examined, 53% of the isolates were *C. albicans*, 13% were *C. famata*, 8% were *C. kefyr*, and 3% were *C. parapsilosis*. In addition, 52% of the samples were determined as blood culture, 20% as wound, 10% as sputum. Of 100 *Candida* strains, 14% were isolated from Neonatal Intensive Care, 14% from General Surgery, and 10% from Internal Intensive Care Units. When the results of this study in terms of species, clinical and clinical sample are compared with other studies, differences are observed. It can be thought that the reasons for this difference may be the duration of hospitalization of the patients, long-term use of antifungal and/or antibiotics, and the differences in nosocomial infection surveillance between hospitals. In our study, the high probability of patients who came at similar time intervals to transmit infection to each other due to a certain period of hospital infection is thought to be the reason for the relatively high incidence of *C. famata* compared to other studies.

In addition, the resistance rates of *Candida* strains examined to antifungal drugs were found to be lower than the results of other studies. Considering the existence of regional differences, it is thought that the resistance rates to antifungal drugs in our study were lower because the intensive care units in our region are fewer than in large centers and *Candida* strains examined have not previously encountered antifungal drugs as a primary infection agent.

When looking at the distribution of clinical samples in which biofilm and hemolytic activities are mostly detected in some studies conducted so far, it is seen that the samples in which biofilm formation is most detected are 40% vaginal secretion, 27% urine, 25% in the study of Yıldırım et al. It was reported that 1% of them were catheters and 18% were blood. It was also reported that 100% of respiratory tract samples, 100% of vaginal samples, 83% of catheters and 82% of urine samples showed hemolytic activity [14]. Satılmış et al., determined biofilm formation in 25%, 13%, 11%, 10%, and 8%, respectively, of *Candida* isolated from blood, general lavage, urine, bronchoalveolar lavage, and sputum [15].

DOI: 10.5455/medscience.2025.04.089

In this study, when the distribution of biofilm formation was examined according to the isolation samples, it was observed that all *Candida* isolated from tracheal aspirate, urine and sputum samples formed biofilms. However, hemolytic activity was seen in *Candida* isolated from 100% of tracheal aspirates, 90% of sputum samples and 88% of urine samples. Although the results are similar to other studies, proportional differences are observed. This proportional difference shows that when planning the treatment of infections caused by strains that cause biofilm and/or hemolysin, the relationship between the species and the biofilm and/or hemolytic activity should be taken into account, rather than the relationship between sample type and biofilm and/or hemolytic activity.

In the study of Alpay et al., the biofilm rate of *C. albicans* was reported as 38%, while this rate was 63% in non-*albicans*. Thus, biofilm production was determined at a rate of 63% in *C. parapsilosis*, 50% in *C. kefyr* and 66% in *C. tropicalis* [13]. According to a study conducted by Melek et al., the biofilm formation rate of *C. albicans* was 10% and the hemolytic activity rate was 95%. In non-*albicans* species, 32% biofilm formation and 88% hemolytic activity were determined [16]. In the study by Yıldırım et al., beta hemolytic activity and biofilm production were found to be 87% and 17%, respectively, in *C. albicans* strains, and 70% and 33%, respectively, in non-*albicans* strains [14]. According to the research of Ekşi et al., *C. albicans* showed 35% biofilm activity while non-*albicans* species showed 65% biofilm activity. However, according to the research of Ekşi et al., *C. tropicalis*, *C. albicans*, *C. dubliniensis* strains showed alpha and beta hemolysis, while *C. famata* and *C. guillermondii* showed alpha hemolysis. Also, *C. krusei*, *C. intermedia*, *C. kefyr*, *C. lusitaniae*, *C.pelliculosa* showed beta hemolysis. However, no hemolytic activity was observed in *C. parapsilosis*, *C. glabrata*, and *C. sake* [5]. Only *C. parapsilosis* strains isolated in a study with *C. parapsilosis* were mostly isolated from blood and catheter, and biofilm formation was reported in 80% of all strains and hemolytic activity in 82% [17]. However, as in our study and similar results of other studies, Neji et al. could not detect biofilm activity in all *C. parapsilosis* strains [5,18]. In a study conducted by Gupta et al. in India, biofilm formation was observed in 49% of *Candida* strains isolated from various clinics and beta hemolysis activity was observed in 24%. It has been reported that *C. tropicalis* has the highest hemolytic activity. While the rate of *C. albicans* producing biofilms was 41%, the rate for non-*albicans* was reported as 54%. Biofilm production rates of *C. tropicalis*, *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. guillermondii*, *C. kefyr* and *C. dublienensis* species respectively, were determined as 58%, 41%, 53%, 50%, 33%, 0%, 50%. While hemolytic activity was determined in *C. tropicalis*, *C. albicans* and *C. parapsilosis* species at the rates of 51%, 47% and 7%, it was not recorded in *C. kefyr*, *C. krusei* and *C. guillermondii* [19]. In another study conducted in China, *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. kefyr*, *C. lusitaniae*, *C. krusei*, *C. tropicalis*, *C. zeylanoides* species showed beta hemolysis, and *C. famata*, *C. guilliermondii*, *C. rugosa*, *C. utilis* species showed alpha hemolysis. *C. parapsilosis*, *C. pelliculosa* species did not show hemolysis [1].

In this study, 57% of a total of 53 *C. albicans* strains isolated from samples from various clinics formed biofilms. Of these, 25% weakly positive, 21% moderately positive, 11% strongly positive biofilm formation was observed. Of the 47 non-*albicans Candida* species, 49% formed a biofilm. Of these, in 15% were recorded weakly positive,15% moderately positive, and 19% strongly positive biofilm formation. Invasive *Candida* infections result from the ability to form biofilms of species. Biofilm is an important virulence factor for *Candida*. Again in this study, 77% of all *C. albicans* strains and 55% of non-*albicans* strains exhibited hemolytic activity. Alpha hemolysis was observed in 28% of *C. albicans* strains and beta hemolysis in 49%. In non-*albicans* strains, 36% alpha hemolysis and 21% beta hemolysis were recorded. The results of this study are similar to the results of other studies.

Antifungal susceptibility testing is of great importance in the treatment of *Candida* infections. In a study conducted in Nepal, 76% of all *C. albicans* strains isolated were Fluconazole Resistant and 50% were resistant to Amphotericin B. It has been reported that there is statistical significance between biofilm formation and fluconazole drug resistance [20]. In a study conducted in Iran, they suggested that there is a significant relationship between fluconazole/itraconazole and biofilm production and between fluconazole and hemolytic activity in *C. albicans* isolates [21]. In this study, it was determined that the sensitivity to antifungals was high in general. As the reason for this, it can be thought that the rate of high positivity in terms of biofilm among *Candida* strains is lower than the rate of weak positivity. When *C. albicans* and non-*albicans* strains were compared in terms of resistance to antifungals, it was determined that non-*albicans* strains were more resistant. Therefore, it can be said that there is a direct correlation between high positive biofilm production and resistance to antifungals in non-*albicans* strains compared to *C. albicans*. Thus, the treatment of infections caused by these emerging resistant strains can also be challenging.

Conclusion

The increase in biofilm and hemolytic activity, which are important virulence and pathogenicity factors for *Candida* species, increases the severity of infections caused by these species. The increase in biofilm formation, especially at the level of strong positive, makes us think that *C. albicans* and non-*albicans* strains increase the infectious feature and the antifungal resistance rate of *Candida*. However, biofilm and hemolytic activity rates of *C. albicans* strains are higher than the rates of non-*albicans* strains showing biofilm and hemolytic activity. Therefore, it is thought that it will increase the incidence of disseminated candidiasis. As a result, it is of great importance to

DOI: 10.5455/medscience.2025.04.089

investigate the prevalence of biofilm formation and hemolysin production among *Candida* strains with multicenter studies and to periodically follow up with its other virulence factors for the prevention and effective treatment of *Candida* infections.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
Ethical approval was obtained for the study from the Afyonkarahisar Health Sciences Clinical Research Ethics Committee (decision no: 2019/200, and date: 14.06.2019). The principles in the Declaration of Helsinki were complied with.

References

1. Luo G, Samaranayake LP, Yau JYY. *Candida* species exhibit differential in vitro hemolytic activities. J. Clin Microbiol. 2001;39:2971-4.
2. Chandra J, Kuhn DM, Mukherjee PK, et al. Biofilm formation by the fungal pathogen *Candida* albicans: development, architecture, and drug resistance. J Bacteriol. 2001;183:5385-94.
3. Yakupoğulları Y, Toraman ZA. Investigation of slime factor Production in *Candida* strains isolated from various clinical samples. Türk Mikrobiyol Cem Derg. 2004;34:178-81.
4. Francolini I, Donelli G. Prevention and control of biofilm based medical-device-related infections. FEMS Immunol Med Microbiol. 2010;59:227-38.
5. Ekşi F, Sözen E, Bayram A, et al. Investigation of slime production and hemolytic activity in *Candida* strains isolated from various clinical samples. Türk Mikrobiyol Cem Derg. 2008;38:27-32.
6. Lamoth F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother. 2018;73:i4-13.
7. Sav H, Baris A, Turan D, et al. The frequency, antifungal susceptibility and enzymatic profiles of *Candida* species in cases of onychomycosis infection. Microb Pathog. 2018;116:257-62.
8. Lohse MB, Gulati M, Johnson AD, Nobile CJ. Development and regulation of single and multi-species *Candida* albicans biofilms. Nat Rev Microbiol. 2018;16:19-31.
9. Yigit N, Aktas E, Dagistan S, Ayyildiz A. Investigating biofilm production, coagulase and hemolytic activity in *Candida* species isolated from denture stomatitis patients. Eurasian J Med. 2011;43:27-32.
10. Sakagami T, Kawano T, Yamashita K, et al. Antifungal susceptibility trend and analysis of resistance mechanism for *Candida* species isolated from bloodstream at a Japanese university hospital. J Infect Chemother. 2019;25:34-40.
11. Brilhante RS, Paiva MA, Sampaio CM, et al. Azole resistance in *Candida* spp. isolated from Catú Lake, Ceará, Brazil: an efflux-pump-mediated mechanism. Braz J Microbiol. 2016;47:33-8.
12. Hasan F, Xess I, Wang X, et al. Biofilm formation in clinical *Candida* isolates and its association with virulence. Microbes Infect. 2009;11:753-61.
13. Alpay Y, Ağalar C, Karabıçak N, et al. Investigation of biofilm formation in *albicans* and non-*albicans Candida* species obtained from clinical specimens and distribution by species. Kocaeli Medical J. 2017;6:23-7.
14. Yıldırım M, Mumcuoğlu İ, Kurşun Ş, et al. Comparison of certain virulence factors in *Candida albicans* and Non-*albicans Candida* strains isolated as infectious agent. Türk Mikrobiyol Cem Derg. 2009;39:62-8.
15. Satılmış ÖK, Akkaya Y, Ergin Ç, Kaleli İ. Slime factor production in *Candida* species isolated from various clinical materials. Pam Med J. 2011;4:25-9.
16. İnci M, Atalay MA, Koç AN, et al. Investigating virulence factors of clinical *Candida* isolates in relation to atmospheric conditions and genotype. Turk J Med Sci. 2012;42:1476-83.
17. Keçeli SA, Kurt M, Özgür M, Otağ ZF. The correlation of biofilm formation, hemolytic and coagulase activites and antifungal susceptibility among *Candida* parapsilosis isolates recovered from clinical speciemens. KOU Sag Bil Derg. 2020;6:203-8.
18. Neji S, Hadrich I, Trabelsi H, et al. Virulence factors, antifungal susceptibility and molecular mechanisms of azole resistance among *Candida* parapsilosis complex isolates recovered from clinical specimens. J Biomed Sci. 2017;24:67.
19. Gupta J, Shah H. In vitro evaluation of virulence factors associated with clinical isolates of *Candida* species from patient in tertiary care hospital in Central India. Tropical Journal of Pathology and Microbiology. 2017;3:188-94.
20. Shrestha BK, Shakya J, Khanal H, et al. Antifungal Susceptibility of Biofilm Producing *Candida* albicans Isolated from Oral Cavity of Diabetic and Non-Diabetic Population of Dharan, Nepal. Preprint from Research Square, 02 Aug 2019. doi: 10.21203/rs.2.12328/v1
21. Mohammadi F, Ghasemi Z, Familsatarian B, et al. Relationship between antifungal susceptibility profile and virulence factors in *Candida* albicans isolated from nail specimens. Rev Soc Bras Med Trop. 2020;53:e20190214.