Vol. 02, No. 02, pp. 59 –63 (2021)
ISSN: 2708-1818

JOURNAL OF LIFE AND BIO-SCIENCES RESEARCH

www.jlbsr.org

Biofilm formation by *Candida albicans* and *Candida glabrata* isolated from urine specimens of diabetic Iraqi women

Enas Abdullah Ibrahim, Rafea Qasim Hamzah*

Department of Biology, College of Education for Pure Science, University of Mosul, Iraq
(enas34ibrahim@gmail.com)(dr.rafeaqm@uomosul.edu.iq)
**Correspondence: dr.rafeaqm@uomosul.edu.iq*

Abstract

Biofilms are multicellular communities where microorganisms are grown and form an extracellular matrix that protects the pathogenic microorganisms from the immunity system and antimicrobial agents. This study is aimed to identify *Candida* spp. isolated from urine specimens by using traditional techniques, germ tube, growth on corn meal agar medium and chromogenic medium then determine the ability of isolates to producing biofilm by tube method (TM) and congo red agar method (CRA). In our study urine specimens were obtained from 174 diabetic females in the period of six months at the Al-Wafa Specialized Center for Diabetes and Endocrinology, Mosul city, Iraq. Out of the total 174 specimens, yeast species were isolated from 56 (32.2 %) specimens. Out of the 56 isolates, 50 isolates were positive for *Candida* spp., especially *C. glabrata* which appeared maximum in 30 isolates (60 %) and followed by *C. albicans* 18 isolates (36%) and *C. krusei* 2 isolates (4 %). In the TM method for biofilm detection, *C. albicans* showed 16 isolates (88.9%) positive for biofilm formation followed by 29 isolates (96.7%) of *C. glabrata*. Furthermore, in the CRA method, all isolates (100%) of *C. albicans* were negative followed by 27 isolates (90%) of *C. glabrata*, whereas only 3 isolates (10%) of *C. glabrata* were positive. We can conclude that TM is the best conventional method and is sensitive to detect biofilm-forming yeast when compared with the CRA method.

Keywords: *Biofilm, Candida albicans, Candida glabrata, Diabetic women*
Received: August 15th, 2021 / Accepted: Oct. 10th, 2021 / Online: Oct. 18th, 2021.

I. INTRODUCTION

Urinary tract infections (UTIs) are a major health concern among both outpatients and hospitalized patients (Goyal *et al*., 2016). It is mainly associated with diabetic patients, because diabetes alters the normal host system, which may contribute to the development of UTI (Woldemariam *et al*., 2019). In recent years, urinary tract infections caused by *Candida* opportunistic pathogens are becoming more frequent and accounted for 10–15% of UTIs (Goyal *et al.,* 2016; Chandak *et al*., 2018; He *et al*., 2021).

The yeast-like fungus *Candida* spp. is the most common cause of a wide range of superficial infections of mucosal surfaces in humans, it colonizes the gastrointestinal and reproductive systems, particularly the female vaginal tract, as well as the skin (Alfouzan and Dhar, 2017). Immunosuppressive disorders such as diabetes, HIV, chemotherapy patients, take broad-spectrum antibiotics or steroids, and indwelling medical equipment implanted in the body such as urinary catheters are all risk factors for invasive *Candida* infections (Devi *et al.,* 2019). There are several factors that may lead to candiduria in diabetic patients such as female gender and uncontrolled diabetes (Geerlings *et al*., 2014).

Urine samples may be positive for *Candida* spp. for a variety of reasons: contamination or colonization, asymptomatic infection or infection with a potential for ascending pyelonephritis, renal and disseminated candidiasis, they may contribute to significant morbidity and mortality, as well as long term hospitalization and increased costs. In most individuals with candiduria there are no traditional symptoms like dysuria, urgency or fever (Alfouzan and Dhar, 2017).

Candida albicans is the most common pathogen that causes *Candida* infections, identified in 50-70% of candiduria cases, and about 20% of candiduria cases are caused by *C. glabrata* (Pieralli *et al*., 2014; Cavalheiro and Teixeira, 2018) Non-*albicans* strains have become more common throughout years, *C. glabrata* is becoming more common in urine specimens across the world and *C. parapsilosis* is prevalent etiology for candidiasis, especially neonatal candiduria (Alfouzan and Dhar, 2017). In the last few decades, a shift towards high-antifungal resistance with non-*albicans Candida* (NAC) infections has been noted (Chandak *et al*., 2018).

doi: 10.38094/jlbsr20246

ipAcademia www.ipacademia.org

Biofilms are complex communities of microorganisms formed and adhered to various types of surfaces, which secrete an exopolymeric substance known as a matrix (Rishabh *et al*., 2017). *Candida* species have the ability to produce biofilm which protects them from the defense of the host immune system and antifungal drugs, and it is one of the particular characteristics of *Candida* pathogenicity (Pakshir *et al.*, 2017; Cavalheiro and Teixeira, 2018), and increased their ability to adhesion on biotic and abiotic surfaces (Thamke *et al*., 2014). In *C. albicans*, hyphal differentiation is a crucial characteristic in biofilm development, influencing its structure and function, whereas *C. glabrata* lacks this capacity, being reported to form pseudo-hyphae (Pereira, 2018).

Recently, clinical infections caused by non-*albicans Candida* (NAC) have been described regularly, and several of these isolates have been shown to produce biofilms (Rishabh *et al*., 2017). The advantages of forming a biofilm include environmental protection, metabolic cooperation, nutrient availability, and the acquisition of recent genetic characteristics (Mohandas and Ballal, 2011).

Biofilms have an essential function as pathogenic agent reservoirs, allowing coinfection with other pathogens, promoting infection persistence, and increasing mortality rates (Pakshir *et al*., 2017). The study objectives were to isolate and identify *Candida* spp. from urine specimens and to detect the ability of *Candida* isolates to biofilm formation. Also, to compare the biofilm formation between *C. albicans* and *C. glabrata* isolates.

II. Materials And Methods

A. *Identification of isolates*

The study was conducted over six months (September 2020 to March 2021), in this study, 174 female diabetic patients were included who attend for diabetic follow up at the Al-Wafa Specialized Center for Diabetes and Endocrinology, Mosul city, Iraq, with ages ranged from 16 to 72 years and who suffer from UTI symptoms. Diabetic patients who have taken any medications, corticosteroids or immunosuppressive therapy during data collection were excluded.

Firstly, clean-catch midstream urine specimens were collected using a sterile, wide-mouth plastic container. Then, the specimens were brought to the laboratory for processing within two hours.

After that, the standard microbiological techniques were used for culturing the specimens on sabouraud dextrose agar (SDA) at 37°C for 24-48 hours of incubation or longer if required. On the other hand, to confirm all doubtful yeast colonies, traditional techniques were used including gram staining, and the germ tube test and chlamydospore formation on cornmeal agar (Forbes *et al*., 2007) For differentiation, these isolates sub-cultured on HiCrome agar medium (HiCrome Candida Differential Agar, HiMedia, India) and incubated at 37°C for 48 hours (Helmy, 2012).

B. *Biofilm Formation*

To detect biofilm formation, two different techniques were employed, as described below:

1. *Tube method*

   The tube method (TM) as described previously by Gokce *et al.* (2007) was used to determine biofilms forming activity, a loopful of each *Candida* isolates grew on SDA plate was inoculated into polystyrene tubes with screw caps, each one containing 10ml of Sabouraud dextrose broth (SDB) with 8% glucose and inoculated tubes have been incubated at 35°C for 48 hours. After incubation, the broth in tubes was aspirated gently and cleaned once with distilled water. After discarding the medium and yeast cells, the tubes were stained with 1% safranin, after 10 minutes, the stain was decanted. To remove any remaining discolouration, the tubes were washed with distilled water. The presence of the visible adherent film lined the wall and the bottom of tubes indicated biofilm formation.

   The test was performed in triplicate and the results were scored as negative –, mild +, moderate ++, and strong +++. Two observers read the results independently (Shin *et al*., 2002).

2. *Congo red agar method*

   For screening isolates that produce biofilm in an alternative technique, congo red agar medium (CRA) used and composed of brain heart infusion broth (BHI) (37 g/L), sucrose (50 g/L), agar (20 g/L), and congo red stain (0.8 g/L) (Janakiram *et al*., 2017). Congo red stain was prepared separately as a concentrated aqueous solution and autoclaved at 121°C for 15 minutes prior to addition separately after the agar had cooled to 55°C. Plates were inoculated and incubated at 37°C for 2-3 days.

   According to Janakiram *et al.* (2017), black colonies with a dry crystalline indicated positive results. The color of a non-biofilm producers colonies is usually pink. The tests were done in triplicate and were repeated three times.

III. Results

In our study of the identification method. Out of the total 174 specimens, yeast species were isolated from 56 (32.2 %) specimen (Figure 1). Out of the 56 isolates, 50 isolates were positive for *Candida* species, majority were *C. glabrata* 30 isolates (60 %) which were the predominant species and followed by 18 isolates (36%) of *C. albicans* and 2 isolates (4 %) of *C. krusei* (Figure 2).

Figure 1: Yeast colonies on SDA plate

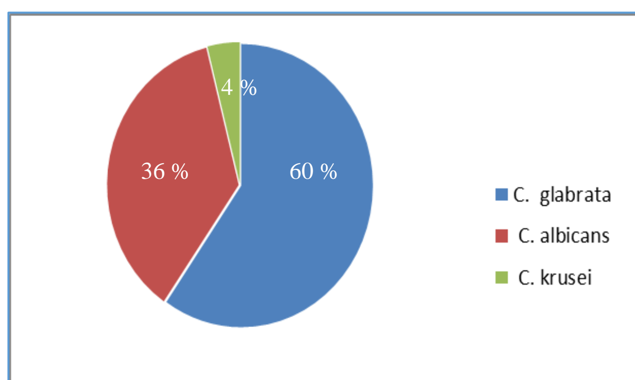

Figure 2: The frequency of *Candida* species isolated from urine of female diabetic patients.

Figure 3: Different *Candida* species on HiCrome Candida differential agar plate. a: *C. albicans*, b: *C. glabrata*, c: *C. krusei*.

Out of 56 yeast isolates, the 18 isolates (positive to germ tube test and chlamydospore formation test) were grown on HiCrome Candida Differential Agar medium in light green color which indicated to the species *C. albicans*. The 38 isolates, 30 of them were grown in cream to white and 2 isolates were grown in purple, fuzzy color which indicated to *C. glabrata* and *C. krusei* respectively, but the 6 of isolates were grown in pink color and this color was not defined by manufacturer instructions (Figure 3). *C. albicans* is detected with a high degree of sensitivity and specificity by this medium, only in rare cases may a *C. albicans* isolate not turn to green color, for this reason, a germ tube test is required for a more certain diagnosis (Reiss *et al*., 2012).

In tube method test for biofilm production, the results have been noted that the number of non-biofilm producers of *C. albicans* were 2 isolates (11.1%), the mild producers were 6 isolates (33.3%), the moderate producers were 10 (55.6%), and no strong producers appeared among *C. albicans* isolates.

Whereas in *C. glabrata* isolates, the results of the number of non-biofilm producers were 1 isolate (3.3%), the mild producers were 3 isolates (10 %), the moderate producers were 20 (66.7%), and 6 isolates (20%) were observed as strong producers (Table 1; Figure 4).

Table 1. Biofilm formation among *Candida* isolates by tube method

Candida spp.	No. of biofilm negative isolates	No. of biofilm positive isolates			Total
		1+	2+	3+	
C. albicans	2	6	10	0	18
C. glabrata	1	3	20	6	30

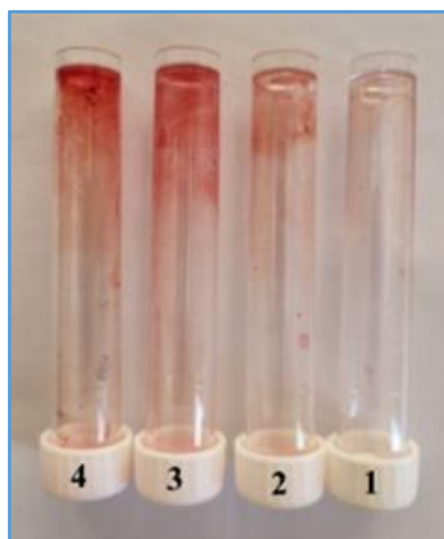

Figure 4: Biofilm formation by tube method 1: Negative, 2: Mild, 3: Moderate, 4: Strong.

In biofilm formation test on congo red agar plates, the results revealed one morphotype for *C. albicans* isolates were 18 isolates (100 %) with pink color colonies which indicated nonbiofilm producers and there are no biofilm producers, while in *C. glabrata* isolates, the results showed two different morphotypes, were 27 isolates (90%) non-producer with pink color colonies and 3 isolates (10%) producers were colonies appeared in black color with crystalline appearance (Table 2; Figure 5).

Table 2. Biofilm formation among *Candida* isolates by congo red agar method.

Candida spp.	No. of biofilm negative isolates	No. of biofilm positive isolates	Total
C. albicans	18	0	18
C. glabrata	27	3	30

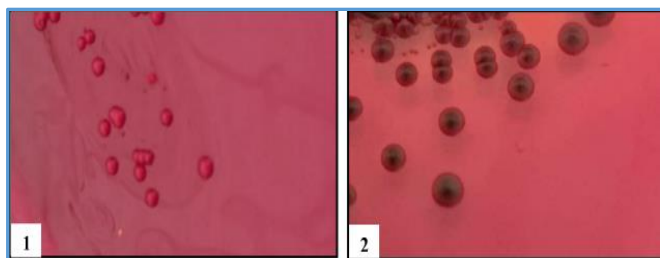

Figure 5: Biofilm formation by tube method 1: Negative isolate, 2: Positive isolate

IV. Discussion

Our results showed that the majority of *Candida* species identified in 50 positive isolates were *C. glabrata* which appeared in 30 isolates (60 %) and followed by *C. albicans* (36%). These results were in agreement with another study showed that *C. glabrata* was the most prevalent *Candida* species etiology of candiduria (50%) in diabetic patients, followed by *C. albicans* (31.6%) and *C. krusei* (10.5%) (Falahati *et al*., 2016). Furthermore, the study of Zakhem *et al*. (2021) showed *C. glabrata* as the predominant species among non-*albicans Candida* in candidemia cases. Recent findings (Sahai and Kumar, 2018; Pramodhini *et al*., 2021) have also been detected that non-*albicans Candida* species were predominant in urinary tract infections. In recent years, we noted that non-*albicans Candida* species prevalence was more than *C. albicans*. Epidemiological data revealed that non-*albicans Candidia* infections are rising globally (Taei *et al*., 2019). Many studies have suggested that the distribution of *Candida* species that cause candiduria varies regionally (Toner *et al*., 2016).

In recent years, we noted that non-*albicans Candidia* species such as *C. glabrata*, the prevalence was more than *C. albicans* which may be ascribed to their potential for adaptation to the conditions of the urinary tract as well as their inherent and/or acquired resistance to conventional antifungals, *C. glabrata* poses a threat to health and a potential cause of therapeutic failure (Falahati *et al*., 2016; Goyal *et al*., 2016).

Results of this study showing majority of the clinical isolates of *C. albicans* and *C. glabrata* exhibiting biofilm-producing abilities by TM methods in contrast with CRA were only 3 isolates of *C. glabrata* biofilm producers similar to that of previous studies of Nerurkar *et al.* (2012) and Chandak *et al*. (2018) which observed highest biofilm activity in non-*albicans Candida* species.

A biofilm is a highly structured community of microorganisms that are embedded in a matrix of polysaccharides linked to one another at a surface or interface (Sardi *et al*., 2014). Biofilm-associated human infections are becoming more common in clinical settings, accounting for 65% to 80% of all infections, biofilm has been identified as a key source of chronic human infections in several investigations using modern microscopy and molecular techniques (Pereira, 2018).

Although the majority of studies have focused on *C. albicans* in the biofilm formation subject, several authors have found that other *Candida* species and other yeast genera, as well as filamentous fungi, are also involved in biofilm development (Sardi *et al*., 2014).

V. Conclusions

Results of our study showed that the majority of *C. albicans* and *C. glabrata* isolates were producers for biofilm by TM method the contrary to CRA methods. As a result, we can conclude that TM is the best conventional method, easy to use and sensitive to detect biofilm-forming yeast whereas the CRA method, is an unreliable method. Furthermore, our results also showed *C. glabrata* is evolving into one of the most prevalent pathogenic yeasts in urinary tract infections of female diabetic patients.

Keeping in mind the high incidence rate of candiduria in people who have diabetes, control of diabetes (especially type 2), risk factors (such as extremes of age and female sex) and causative connections between diabetes and candiduria should all be emphasized.

Conflict Of Interest

The authors declare no conflict of interest

References

Alfouzan, W.A., Dhar, R. (2017). Candiduria: evidence-based approach to management, are we there yet?. *Journal de mycologie medicale*, 27(3), 293-302.

Cavalheiro, M., Teixeira, M.C. (2018). Candida biofilms: threats, challenges, and promising strategies. *Frontiers in medicine*, *5*, 28.

Chandak, R.J., Mishra, B., Thakur, A., Loomba, P., Dogra, V. (2018). Comparison of methods of biofilm detection in urinary Candida isolates and evaluating its role in persistent candiduria. *National Journal of Laboratory Medicine*, 7(2), 60-116.

Devi, A.R., Hymavathi, R., Mounika, G. (2019). Candida Species Isolation, Identification and Biofilm Detection at a Tertiary Care Hospital. *Int J Cont Med Res*, 6(4),45.

Falahati, M., Farahyar, S., Akhlaghi, L., Mahmoudi, S., Sabzian, K., Yarahmadi, M., Aslani, R. (2016). Characterization and identification of candiduria due to Candida species in diabetic patients. *Current medical mycology*, 2(3), 10.

Forbes, B.A., Sahm, D.F., Weissfeld, A.S. (2007). Bailey and Scott's Diagnostic Microbiology. 12th ed., Mosby, Elsevier, Inc., U.S.A, pp. 710 -711.

Geerlings, S., Fonseca, V., Castro-Diaz, D., List, J., Parikh, S. (2014). Genital and urinary tract infections in diabetes: impact of pharmacologically-induced glucosuria. *Diabetes research and clinical practice*, 103(3), 373-381.

Gokce, G., Cerikcioglu, N., Yagci, A. (2007). Acid proteinase, phospholipase, and biofilm production of Candida species isolated from blood cultures. *Mycopathologia*, 164(6), 265-269.

Goyal, R. K., Sami, H., Mishra, V., Bareja, R., Behara, R.N. (2016). Non-albicans candiduria: an emerging threat. *J Appl Pharm Sci*, 6(3), 48-50.

He, Z., Huo, X., Lei, D., Zhao, H., Jia, K., Wang, F. (2021). Management of candiduria in hospitalized patients: a single-center study on

the implementation of IDSA guidelines and factors affecting clinical decisions. *European Journal of Clinical Microbiology & Infectious Diseases*, 40(1), 59-65.

Helmy, M.M. (2012). Phenotypic analysis of Candida Species Associated with Vulvovaginal Candidiasis. *Egypt J Med Microbiol*, 21(1), 109-115.

Janakiram, B., Myneni, R.B., Kumar, K.A., Gousia, Sk., Latha, J.N.L. (2017). Methods of Determination of Biofilm Formation by *Candida albicans*. *Res. J. Microbiol.,* 12(1): 90-96.

Mohandas, V., Ballal, M. (2011). Distribution of Candida species in different clinical samples and their virulence: biofilm formation, proteinase and phospholipase production: a study on hospitalized patients in southern India. *Journal of Global Infectious Diseases*, 3(1), 4.

Nerurkar, A., Solanky, P., Chavda, N., Baria, H., Desai, B. (2012). Isolation of Candida Species in clinical specimens and its virulence factor: The biofilm. *International Journal of Medical Science and Public Health*, 1 (2), 97-100.

Pakshir, K., Bordbar, M., Zomorodian, K., Nouraei, H., Khodadadi, H. (2017). Evaluation of CAMP-like effect, biofilm formation, and discrimination of *Candida africana* from vaginal *Candida albicans* species. *Journal of pathogens*, 2017.

Pereira, D.G. (2018). Biofilm formation in *Candida glabrata*: the role of the Transcription Factor Tec1. M.Sc. thesis, Tecnico School, Lisboa, Portugal.

Pieralli, F., Bazzini, C., Vannucchi, V., Mancini, A., Nozzoli, C. (2014). A case of *Candida glabrata* severe urinary sepsis successfully treated with micafungin. *Medical mycology case reports*, 5, 1-3.

Pramodhini, S., Srirangaraj, S., Easow, J.M. (2021). Candiduria-Study of Virulence Factors and Its Antifungal Susceptibility Pattern in Tertiary Care Hospital. *Journal of Laboratory Physicians*, 13(3), 231–237.

Reiss, E, Shadomy, H.J., Lyon, G.M. (2012). Fundamental Medical Mycology. John Wiley and Sons, Inc., USA. p. 44.

Rishabh, V., Jain Pavithra, K.R., Kulkarni, R.D. (2017). Pathogenicity Testing of Clinical Candida Isolates by Assessing Biofilm Formation and their Adhesion to Urinary Catheter Material. *Journal of Pure and Applied Microbiology*, 11(4), 1939-1946.

Sahai, S., Kumar, A. (2018). Role of Candida in Catheter Associated Urinary Tract Infection. *Int J Cur Res Rev*, 10(20), 15-9.

Sardi, J.D.C.O., Pitangui, N.D.S., Rodríguez-Arellanes, G., Taylor, M.L., Fusco-Almeida, A.M., Mendes-Giannini, M.J.S. (2014). Highlights in pathogenic fungal biofilms. *Revista iberoamericana de micologia*, 31(1), 22-29.

Shin, J.H., Kee, S.J., Shin, M.G., Kim, S.H., Shin, D.H., Lee, S. K., Ryang, D.W. (2002). Biofilm production by isolates of Candida species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. *Journal of Clinical Microbiology*, 40(4), 1244-1248.

Taei, M., Chadeganipour, M., Mohammadi, R. (2019). An alarming rise of non-albicans Candida species and uncommon yeasts in the clinical samples; a combination of various molecular techniques for identification of etiologic agents. *BMC research notes,* 12(1), 1-7.

Thamke, D.C., Tayade, H.A., Tayade, S.A. (2014). Biofilm formation among Candida albicans isolated from vagina. *IJBAR*, 20, 05-12.

Toner, L., Papa, N., Aliyu, S.H., Dev, H., Lawrentschuk, N., Al-Hayek, S. (2016). *Candida* growth in urine cultures: a contemporary analysis of species and antifungal susceptibility profiles. QJM: *An International Journal of Medicine*, 109(5), 325-329.

Woldemariam, H.K., Geleta, D.A., Tulu, K.D., Aber, N.A., Legese, M.H., Fenta, G.M., Ali, I. (2019). Common uropathogens and their antibiotic susceptibility pattern among diabetic patients. *BMC infectious diseases*, 19(1), 1-10.

Zakhem, A.E., Istambouli, R., Alkozah, M., Gharamti, A., Tfaily, M.A., Jabbour, J.F., Araj, G.F., Tamim, H., Kanj, S.S. (2021). Predominance of *Candida glabrata* among non-albicans Candida species in a 16-year study of candidemia at a tertiary care center in Lebanon. *Pathogens*, 10(1), 82.