Int.J.Curr.Microbiol.App.Sci (2022) *11*(07): 24-33

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 07 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1107.004

Impact of Quorum Sensing Molecules at Supra-physiological Concentrations on Virulence Factors of Multiple Drug Resistant *Candida* Clinical Isolates

Tarek El-Said El-Banna, Fatma Ibrahim Sonbol, Sahar Nasser Al-Kharsawy* and Lamiaa A. Al-Madboly

Department of Pharm. Microbiology, Faculty of Pharmacy, Tanta University, Tanta, Egypt

**Corresponding author*

Keywords

Candida albicans, virulence, Farnesol, sap5, hwp1

Article Info

Received:
06 June 2022
Accepted:
28 June 2022
Available Online:
10 July 2022

A B S T R A C T

Candida albicans a polymorphic fungus which have the ability to develop as yeast, pseudohyphae and/or true hyphae forms. *Candida* isolates (n=34) were collected from different clinical specimens and identification to the species level was performed. Isolates were subjected to antifungal susceptibility testing against (nystatin, amphotericinB, Itraconazole, fluconazole, terbinafine, and caspofungin) and determination of their minimum inhibitory concentrations (MICs) were carried out according to the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2015) strategies. Virulence factors including secreted hydrolases (phospholipase and aspartyl protease) and biofilm production were evaluated. The hyphal form of *Candida* is themain role in infection process during its invasion to mucosal membrane. A group of genes participate in monitoring of hyphae formation in *C. albicans*, including*sap5* and *hwp1*.Farnesol compound is a quorum sensing molecule which prevents converting of yeast-to-hyphae form. The aim of our study was to determine farnesol effect in yeast-to-hyphae shift, and its related gene expressions in *C. albicans* using qRT-PCR. Real time-PCR analysis revealed that 1/2 MIC of farnesol has significantly decreased the expressionof*sap5*and *hwp1*genes in comparison to control group ($p<0.05$).

Introduction

Candida albicans is one of the normal flora present in human, inhabiting the oral cavity, the genitourinary and gastrointestinal tracts in most of people with healthy state. The shift from commensal to a pathogen between *Candida* spp. is related to many virulence factors such as tissue adhesion, secretion of hydrolases and biofilm production (Mayer *et al.*, 2013). Secreted phospholipases break ester bonds in glycerol-phospholipids causing destruction in the membrane of host-cell and participate in host tissue adherence (Keyhani *et al.*, 2018). Secreted aspartyl proteases (Saps) enable the host tissue adhesion and damage and contribute in alterations in the immune evasion of the host (Monika *et al.*, 2017). Biofilms are extremely organized populations of microorganisms which are

attached to the host surface and surrounded by a self-produced extracellular matrix. Biofilms are important for protection of fungal development and surviving in aggressive environmental conditions (Ramage *et al.,* 2012). A group of genes play a role in monitoring the hyphae formation in *Candida* including *sap5* (Secreted Aspartyl Proteinase 5), *hwp1*(Hyphal WallProtein 1) (Nikoomanesh *et al.,* 2018).

The existing antifungal therapy for curing infections of *Candida* is limited to four main classes of antifungal drugs, comprising the most commonly prescribed azoles, polyenes, allyl-amines and the recently generated echino-candins, that can be used as an alternative treatment for isolates displaying resistance to the above-mentioned antifungal drugs (Cowen *et al.,* 2015).The antifungal drug resource is still limited although recent drugs have been produced in latest years. This has led to looking for new compounds that own antifungal properties (Cordeiro *et al.,* 2013). Furthermore, the treatment disrupting quorum sensing is an alternative approach. The use of supra-physiological concentration of quorum sensing molecules may adversely affect the cell-to-cell communication in biofilms (Kovács *et al.,* 2020). Quorum sensing molecules play a vital role in morphogenesis, biofilm production, control of nutrient competition and limitation of cell population, and are important for the infectious process, especially for the distribution and formation of colonies at different locations (Wongsuk *et al.,* 2016).

These molecules regulate gene expression, cellular differentiation, and other functions. Among the quorum sensing molecules, farnesol and tyrosol released by *Candida* species such as *C. albicans* have received attention for presenting anti-biofilm activity and studying their effect on the acid production and hydrolytic enzymes (phospholipase and aspartyl proteinase).

The current study aim was to evaluate the *in vitro* antifungal susceptibility profiles, the secretion of hydrolytic enzymes (phospholipase and aspartyl protease) and biofilm formation, as well as determination of farnesol concentration effect on inhibiting formation of hyphae.

Materials and Methods

Fungal isolates

In our study, *C. albicans* isolates (n=34) were collected from different clinical specimens, recovered from different patients from various departments of Tanta University Teaching Hospitals (Egypt) between 2016 and 2017. Identification of isolates to the species level was performed using CHROMagar *Candida* (HiCrome *Candida* Differential Agar – HiMedia Laboratories, India). Confirmation of identity was carried our using MALDI-TOF/MS. Stock cultures were preserved in epindorff containing glycerol broth at -80°c. All *Candida* isolates were sub-cultured on SDA at 28 °C for 24-48 hPrior to being tested to confirm purity and viability.

Antifungal resistance profile

In vitro antifungal susceptibility testing was performed according to the recommendations proposed by the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2015) guidelines. Amphotericin B (AMB), nystatin (NYS), Itraconazole (ITRA), terbinafine (TER), Fluconazole (FLU) and caspofungin (CAS) were used in the susceptibility testing. RPMI-1640 medium with L-glutamine/without bicarbonate (Oxoid, UK), buffered with 0.165M 3-N-morpholinepropanesulfonic acid (MOPS), pH 7.0, was used for the broth microdilution test. The minimum inhibitory concentrations (MICs) were determined according to EUCAST, 2015 recommendations guidelines.

Susceptibility testing of quorum sensing compounds

The prepared concentrations of farnesol ranged from 0.29 – 150 µM as previously described by Cordeiro

et al., (2013). Tyrosol was tested at concentrations varying from 42.96 to 22000µM, which are approximately 2× to 1000× the concentration reached by endogenous tyrosol in *C. albicans* (Cordeiro *et al.,* 2015).

Germ tube test (GTT)

Human serum (0.5 mL) was transferred to a Wassermann tube using a sterile pipette, one colony of freshly sub-cultured *Candida* on SDA was gently emulsified into the serum, the tube was incubated at 37°C for 3 h only. After that a drop of the serum was transferred to a slide for examination under light microscope oil immersion lens (100x) (Madhavan *et al.,* 2011).

Phospholipase assay

The activities of phospholipase were assessed as previously described by Al-Abeid *et al.,* (2004). The phospholipase index (P_z) was interpreted by dividing the ratio of the diameter of the colony on the total diameter of the colony plus the precipitation zone. The lower was the P_z value for each isolate, the higher would be the phospholipase activity.

Aspartyl protease assay

Aspartyl proteinase activities of *C. albicans* isolates were estimated as previously described by Al-Abeid *et al.,* (2004). The proteinase index (Pr_z) was calculated by dividing the ratio of the colony diameter on that of the unstained zone of proteolysis. A Pr_z, value of 1 reveals negative protease activity; Pr_z, <1 means aspartic proteinase production by the isolate. The aspartic proteinase activity was higher when the Pr_z, value was lower (Deorukhkar *et al.,* 2014).

Screening for biofilm formation

Biofilm production was screened using the microtiter plate method as previously described by Jin *et al.,* (2003). *C.albicans* ATCC 90028 was used as a positive control for biofilm production. The OD values were recorded for all tested isolates and negative controls. The isolates were then classified as non- biofilm producers when OD_{590} nm ≤ ODc, weak biofilm producers, when ODc < OD_{590} nm ≤ 2×ODc, moderate biofilm producers, when 2×ODc< OD_{590} nm ≤ 4×ODc, and strong biofilm producers when OD_{590} nm >4× ODc (Brilhante *et al.,* 2017).

Real-Time PCR

Real-time PCR was used to measure the effect of farnesol quorum sensing on expression of genes *sap 5* and *hwp 1* in three selected isolates. Expression of the target genes in both treated and untreated isolates was measured.

The target genes expression was normalized to the reference gene *act1*expression. Calculation of fold increase and fold decrease in gene expression were calculated according to the following equations (Livak and Schmittgen, 2001):

ΔCt = Ct (Target A treated) _ Ct (Ref B treated)

ΔCt = Ct (Target A control) _ Ct (Ref B control)

ΔΔCt = ΔCt (treated)_ ΔCt control

Normalized target gene expression level= $2^{-\Delta\Delta CT}$

Results and Discussion

All isolates of *C. albicans* produced green colonies after cultivation on *Candida* Chromogenic agar as shown in Figure 1. Confirmation of identity was carried out using MALDI-TOF MS.

Minimum inhibitory concentrations (MICs) values of different antifungal agents against *C. albicans* isolates are recorded in Table 1. All isolates were sensitive to CAS (MIC ≤ 1µg/mL), NYS (MIC ≤ 64µg/mL), while 70.6 % were susceptible to ITRA (MIC ≥ 1µg/mL). Moreover, the isolates showed high resistance levels to the TER, AMP and GRIS antifungal agents tested. Concerning TER (MIC ≥8 µg/mL), AMP (MIC ≥ 2µg/mL) and FLU (MIC ≥

8µg/mL), the isolates showed 58.8, 73.5, 88.2% resistance to the antifungal agents, respectively.

The MICs of farnesol varied from 0.29– 150 µ M for all *candida* isolates while the MICs for tyrosol ranged from 2750 to 5500µM as shown in Table 2.

All *C. albicans* isolates produced germ tubes up on examination by light microscope. Representative picture of germ tube formation under microscope is shown in Fig.2.

Concerning extracellular hydrolytic activities, phospholipase production appeared in the form of precipitation zone around *Candida* species on egg yolk medium as shown in Fig.3. Approximately 47.1% were able to produce phospholipase as presented in Table 3.

Aspartyl protease activity appeared as precipitation zone around *Candida* spp. as shown in Fig.3. It was noticed in 82.3% of the tested isolates as shown in Table 4.

Screening of all isolates for biofilm production was carried out using microtitre plate method. Furthermore, *C. albicans* isolates were categorized according to their abilities of biofilm production into strong (n=30; 88.2%), moderate (n=2; 5.9%), Weak (n=2; 5.9%) and non-biofilm producers (n=0, 0%).

The effect of farnesol on gene expression in *C. albicans* isolates using real-time PCR testing

The expression of the *hwp1* and *sap5* genes that are responsible for biofilm formation and protease production were evaluated in 3 clinical isolates of *C. albicans* before and after treatment with farnesol using real-time PCR testing. Farnesol at 1/2 MIC caused a marked decline in the expression values of both *hwp1* and *sap5* genes compared to untreated isolates. The t-test was used to compare the control group and 1/2 MIC farnesol treated group to assess whether there were significant differences between both of them in regard to effect on gene expression. It was found that all the virulence genes of the tested 3 isolates were significantly (< 0.001) down-regulated after treatment with farnesol.

Antifungal drugs available for clinicians seem to be diverse. However, few classes of antifungal agents are currently available as therapeutic options for mucosal or systemic candidiasis (Bersani *et al.*, 2019). The results revealed that caspofungin produced the maximum activity against all tested *Candida albicans*. Similar results were reported by Amanloo *et al.,* (2018) who reported who stated that all isolates were susceptible to caspofungin. Therefore, caspofungin is recommended with confidence in the treatment of infections occurring with *Candida albicans*. However, it should be used wisely to avoid development of resistance.

In the present study, the attained antifungal susceptibility pattern revealed that *C. albicans* isolates were highly sensitive to NYS (100%). These results are similar to the studies reported by El-Houssaini *et al.,* (2019). Regarding the antifungal activity of amphotericin B, the data of our study revealed that MIC values of 73.5% of tested isolates showed resistance to amphotericin B with MIC values of 2-16 µg/ml. Comparable results were reported by Soliman *et al.,* (2021) where 50% of isolates showed resistance to amphotericin B. However, Eksi *et al.,* (2013) reported that all *Candida* isolates were sensitive to amphotericin B.

Difference of amphoteicin B resistance rates in different series may be caused by variation in patient population, distribution of *Candida* spp., previous use of antifungal agents, methods of *in vitro* susceptibility testing, and different breakpoint values of amphoteicin B (Metin *et al.,* 2011).

Data obtained in this study showed resistance of *Candida albicans* to fluconazole (88.2%). El-Houssaini *et al.,* (2019) in Egypt recorded higher resistance to azoles which reached up to 98.5% resistance in case of fluconazole. This high resistance to azole drugs might be explained by the common use of azole antifungal agents for the curing of cutaneous and oral candidiasis.

Table.1 Minimum inhibitory concentrations (MICs) values of different antifungal agents against *C. albicans* isolates.

Antifungal drug (break point conc. in µg/ml)*	MIC (µg/ml)	No. of isolates	Antifungal drug (break point conc. in µg/ml)*	MIC (µg/ml)	No. of isolates
Amphotericin B (≥ 2)	64	-	Caspofungin (≥ 1)	2	-
	32	-		1	-
	16	2		0.5	-
	8	10		0.25	-
	4	7		0.125	-
	2	6		0.06	-
	1	9		0.03	5
	0.5	-		0.015	3
	0.25	-		0.007	2
	0.125	-		0.003	24
Itraconazole (≥ 1)	32	-	Fluconazole (≥ 8)	> 256	10
	16	-		128	6
	8	-		64	3
	4	2		32	4
	2	8		16	4
	1	7		8	3
	0.5	9		4	3
	0.25	8		2	1
	0.125	-		1	-
	0.06	-		0.5	-
	0.03	-		< 0.5	-
Nystatin (≥ 64)	>1024	-	Terbinafine (≥ 8)	> 256	10
	512	-		128	1
	256	-		64	1
	128	-		32	5
	64	-		16	3
	32	1		8	-
	16	1		4	3
	8	2		2	2
	4	11		1	1
	2	2		0.5	-
	< 2	17		< 0.5	8

Table.2 MICs values of tested quorum sensing compounds against selected MDR isolates.

Quorum sensing compound	No. of *Candida* species (%) inhibited by the tested quorum sensing compounds at different conc. (µM)									
Farnesol	**150**	**75**	**37.5**	**18.75**	**9.37**	**4.68**	**2.34**	**1.17**	**0.58**	**0.29**
	2 (5.8%)	-	4 (11.7%)	8 (23.5%)	1 (2.9%)	6 (17.6%)	-	-	1 (2.9%)	**12 (35.2%)**
Tyrosol	**22000**	**11000**	**5500**	**2750**	**1375**	**687.5**	**343.8**	**171.9**	**85.9**	**42.96**
	-	-	11 (32.3%)	23 (67.6%)	-	-	-	-	-	-

Table.3 Production of phospholipase enzyme by tested *Candida* spp.

Candida species	Phospholipase activity (Pz)*	No. of isolates showing phospholipase activity (%)**
C. albicans **(n= 34)**	-	18 (52.9)
	+	7 (20.6)
	++	6 (17.6)
	+++	2 (5.9)
	++++	1 (2.9)

*P_z,phospholipase activity zone. {-: negative, P_z =1; +: Weak positive, P_z = 0.90-0.99; ++: medium positive, P_z = 0.80-0.89; +++: strong positive, P_z = 0.70-0.79; ++++: very strong, P_z = <0.70}.
**Percent calculated relative to the corresponding species.

Table.4 Production of aspartyl protease enzyme by tested *Candida* spp.

Candida species	Protenaise activity (Prz)*	No. (%) of isolates showing proteinase activity **
C. albicans **(n= 34)**	-	6 (17.6)
	+	10 (29.4)
	++	13 (38.2)
	+++	2 (5.88)
	++++	3 (8.8)

*Pr_z, proteinase activity zone. {-: negative, Pr_z=1; +: Weak positive, Pr_z= 0.90-0.99; ++: medium positive, Pr_z= 0.80-0.89; +++: strong positive, Pr_z= 0.70-0.79; ++++: very strong, Pr_z= <0.70}.
**Percent calculated relative to the corresponding species.

Fig.1 Growth of *Candida* isolates on CHROM agar

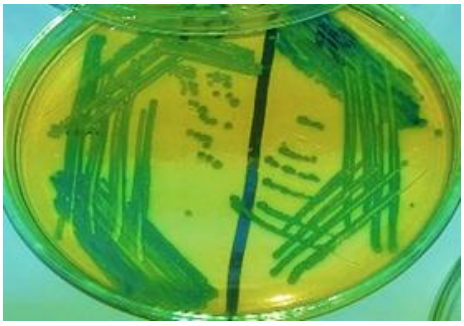


Fig.2 Germ tube formed by *Candida albicans* isolate under light microscope (100 x) (a) compared to negative control (b)

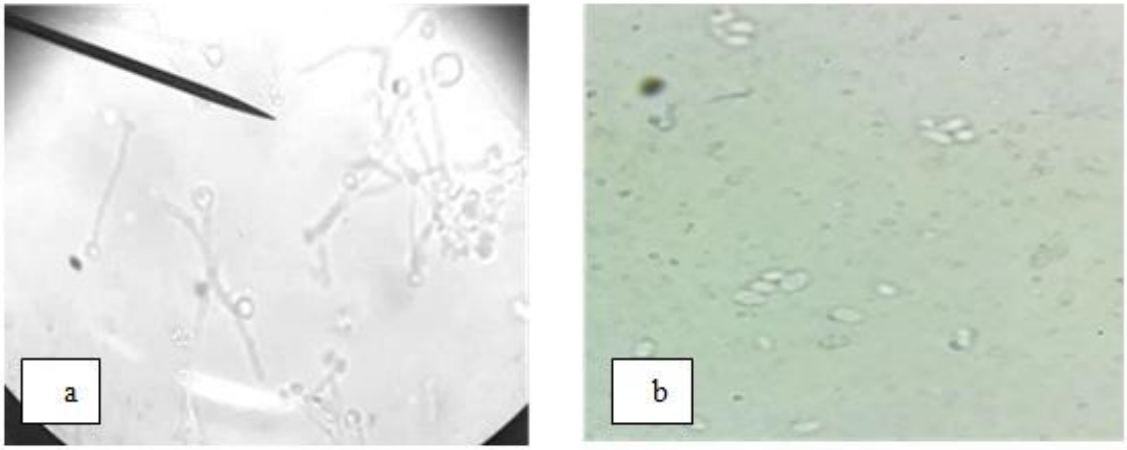


Fig.3 Phospholipase precipitation zone around Candida species (a) compared to ATCC 90028 strain negative control (b) on egg yolk agar medium.

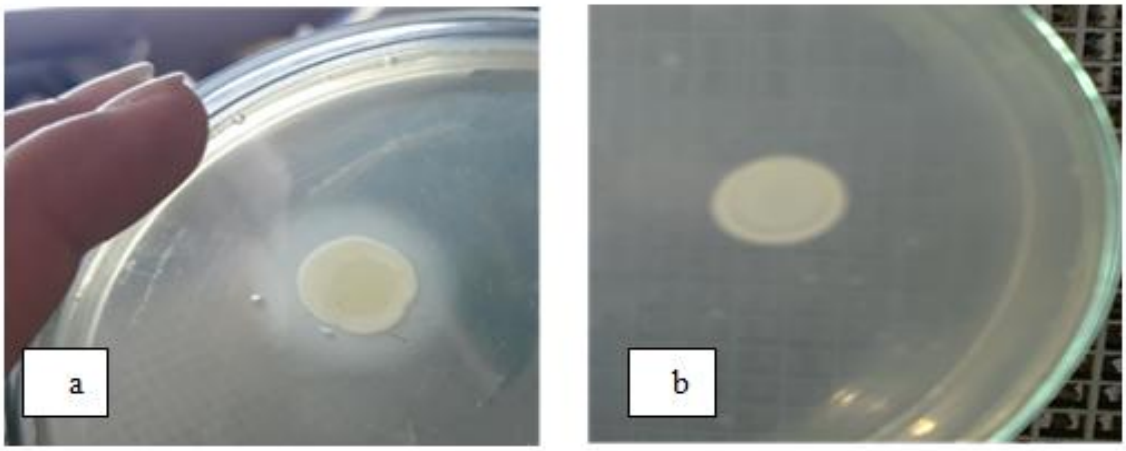
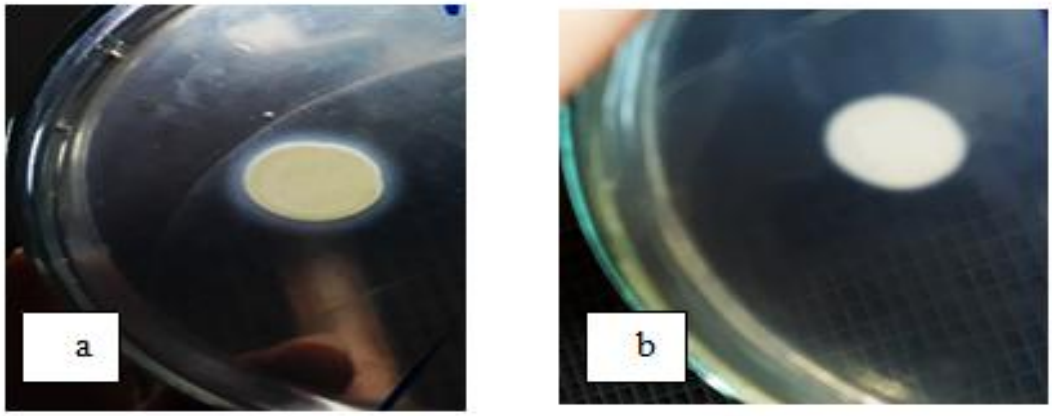


Fig.4 Sabouraud Dextrose Agar containing BSA and showing growth of *Candida albicans* isolate with precipitation zone around the colonies (a), compared to negative control (b).



The wide use of fluconazole in treatment and prophylaxis is the main factor responsible for the development of its resistance (Mashaly *et al.,* 2019). On the other hand, 70.6% of all tested *Candida albicans* isolates were susceptible to Itraconazole. Ghaith *et al.,* (2021) reported (71.4 %) resistance of isolates to itraconazole.

In our study, the MIC values of these quorum-sensing compounds were determined and found to be ranged between 0.29 and 150µM for farnesol while those of tyrosol ranged between 2750 and 5500µM indicating higher potency of farnesol relative to tyrosol. This finding was consistent with the results of Cordeiro *et al.,* (2013) and Cordeiro *et al.,* (2015) in Brazil.

In this study, germ tube formation was found to be positive in all *C. albicans* isolates. This was supported by several workers (El-Houssaini *et al.,* 2019 and Soliman *et al.,* 2021) as well as Shiyamalee *et al.,* (2020). Data obtained in our study recorded that 47.1 % of *C. albicans* isolates were phospholipase producers.

Former studies have recorded the rate of phospholipase production in *C. albicans* to be 47.6% (Udayalaxmi *et al.,* 2016), 81.08 % (Fule *et al.,* 2015) and 100% (de Souza Ramos *et al.,* 2015). Arslan *et al.,* (2016) reported that high secretion of phospholipase is related to advanced degree of pathogenicity where phospholipases cause lipids digestion, and inflammatory processes initiation (Nikou *et al.,* 2019). Our results also revealed that the majority (>50%) of the tested isolates were positive for aspartyl proteinase production. Also in Egypt, El-Houssaini *et al.,* (2019) mentioned that approximately 40 % of *C. albicans* isolates were capable of producing proteinase enzyme and Mohammadi *et al.,* (2021) reported that 72.85% of *C. albicans* isolates secreted aspartyl proteinase.

The data obtained in our study revealed that strong biofilm production was detected in almost 88 % of *C. albicans* isolates. Gharaghani *et al.,* (2021) study revealed that 100% of *C. albicans* were biofilm producers. On the contrary, the study of El-Houssaini *et al.,* (2019) revealed that *C. albicans* isolates had the least (3.1%) prevalence of biofilm development. This controversy might be explained by the fact that the selection and origin the of isolates can influence biofilm formation in *Candida* species.

In our study, RT-PCR technique was used to evaluate the effect of the tested quorum sensing compound; farnesol on*hwp1* and *sap5* virulence genes expression. The results revealed high potency of farnesol that significantly ($p<0.001$) down-regulated the expression of aforementioned genes at

concentration as low as 18.75 µM in all tested isolates compared to their corresponding controls. A similar study performed by Nikoomanesh *et al.*, (2019) who reported that farnesol at concentration of 300µM caused significant decrease in the expression of both *hwp1* and *sap5*genes.

References

Al-Abeid, H. M., Abu-Elteen, K. H., Elkarmi, A. Z., & Hamad, M. A. (2004). Isolation and characterization of *Candida* spp. in Jordanian cancer patients: prevalence, pathogenic determinants, and antifungal sensitivity. Jpn J Infect Dis, 57(6), 279-284.

Amanloo, S., Shams-Ghahfarokhi, M., Ghahri, M., & Razzaghi-Abyaneh, M. (2018). Drug susceptibility profile of *Candida glabrata* clinical isolates from Iran and genetic resistant mechanisms to caspofungin. Revista Iberoamericana de Micología, 35(2), 88-91.

Arslan, S., Koç, A. N., Şekerci, A. E., Tanriverdi, F., Sav, H., Aydemir, G., & Diri, H. (2016). Genotypes and virulence factors of *Candida* species isolated from oralcavities of patients with type 2 diabetes mellitus. *Turkish journal of medical sciences*, *46*(1), 18-27.

Bersani, I., Piersigilli, F., Goffredo, B. M., Santisi, A., Cairoli, S., Ronchetti, M. P., & Auriti, C. (2019). Antifungal drugs for invasive candida infections (ICI) in neonates: Future perspectives. *Frontiers in pediatrics*, 7, 375.

Brilhante, R. S. N., Bittencourt, P. V., de Souza Collares Castelo-Branco, D., de Melo Guedes, G. M., de Oliveira, J. S., Alencar, L. P.,... & Rocha, M. F. G. (2017). Biofilms of *Candida* spp. from the ocular conjunctiva of horses with reduced azole susceptibility: a complicating factor for the treatment of keratomycosis?. *Veterinary ophthalmology*, *20*(6), 539-546.

Cordeiro, R. A., Teixeira, C. E., Brilhante, R. S., Castelo-Branco, D. S., Paiva, M. A., Giffoni Leite, J. J.,... & Rocha, M. F. (2013). Minimum inhibitory concentrations of amphotericin B, azoles and caspofungin against *Candida* species are reduced by farnesol. *Medical mycology*, *51*(1), 53-59.

Cordeiro, R. D. A., Teixeira, C. E., Brilhante, R. S., Castelo-Branco, D. S., Alencar, L. P., de Oliveira, J. S.,... & Rocha, M. F. (2015). Exogenous tyrosol inhibits planktonic cells and biofilms of *Candida* species and enhances their susceptibility to antifungals. *FEMS yeast research*, *15*(4), fov012.

Cowen, L. E., Sanglard, D., Howard, S. J., Rogers, P. D., & Perlin, D. S. (2015). Mechanisms of antifungal drug resistance. *Cold Spring Harbor perspectives in medicine*, *5*(7), a019752.

de Souza Ramos, L., Barbedo, L. S., Braga-Silva, L. A., Dos Santos, A. L. S., Pinto, M. R., & da Graça Sgarbi, D. B. (2015). Protease and phospholipase activities of *Candida* spp. isolated from cutaneous candidiasis. *Revista iberoamericana de micologia*, *32*(2), 122-125.

Deorukhkar, S., & Saini, S. (2014). Virulence markers and antifungal susceptibility profile of *Candida glabrata*: An emerging pathogen. *Microbiology Research Journal International*, 39-49.

Eksi, F., Gayyurhan, E. D., & Balci, I. (2013). In vitro susceptibility of *Candida* species to four antifungal agents assessed by the reference broth microdilution.

El-Houssaini, H. H., Elnabawy, O. M., Nasser, H. A., & Elkhatib, W. F. (2019). Correlation between antifungal resistance and virulence factors in *Candida albicans* recovered from vaginal specimens. *Microbial pathogenesis*, *128*, 13-19.

Fule, S. R., Das, D., & Fule, R. P. (2015). Detection of phospholipase activity of *Candida albicans* and non albicans isolated from women of reproductive age with vulvovaginal candidiasis in rural area. *Indian Journal of Medical Microbiology*, *33*(1), 92-95.

Ghaith, D., Zafer, M. M., Hosny, T., & AbdElfattah, M. (2021). MALDI-TOF MS Overcomes Misidentification of the Uncommon Human Pathogen *Candida famata* by Routine Phenotypic Identification Methods. *Current Microbiology*, *78(4)*, 1636-1642.

Gharaghani, M., Rezaei-Matehkolaei, A., Hardani, A. K., & Mahmoudabadi, A. Z. (2021). Pediatric candiduria, epidemiology, genotype distribution and virulence factors of *Candida albicans*. *Microbial Pathogenesis*, *160*, 105173.

Jin, Y. Y. H. K., Yip, H. K., Samaranayake, Y. H., Yau, J. Y., & Samaranayake, L. P. (2003). Biofilm-forming ability of *Candida albicans* is unlikely to contribute to high levels of oral yeast carriage in cases of human immunodeficiency virus infection. *Journal of clinical microbiology*, *41*(7), 2961-2967.

Keyhani, N. O. (2018). Lipid biology in fungal stress and virulence: Entomopathogenic fungi. *Fungal biology*, *122*(6), 420-429.

Kovács, R., & Majoros, L. (2020). Fungal quorum-sensing molecules: a review of their antifungal effect against *Candida* Biofilms. *Journal of Fungi*, *6*(3), 99.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods*, *25*(4), 402-408.

Madhavan, P., Jamal, F., Chong, P. P., & Ng, K. P. (2011). Identification of local clinical *Candida* isolates using CHROMagar *Candida* TM as a primary identification method for various Candida species. *Tropical biomedicine*, *28*(2), 269-274.

Mashaly, G., & Shrief, R. (2019).*Candida glabrata* complex from patients with healthcare-associated infections in Mansoura University Hospitals, Egypt: Distribution, antifungal susceptibility and effect of fluconazole and polymyxin B combination. *Germs*, *9(3)*, 125.

Mayer, F. L., Wilson, D., & Hube, B. (2013). *Candida albicans* pathogenicity mechanisms. *Virulence*, *4*(2), 119-128.

Metin, D. Y., Hilmioglu-Polat, S., Samlioglu, P., Doganay-Oflazoglu, B., Inci, R., & Tumbay, E. (2011). Evaluation of antifungal susceptibility testing with microdilution and Etest methods of Candida blood isolates. Mycopathologia, 172(3), 187-199.

Mohammadi, F., Hemmat, N., Bajalan, Z., & Javadi, A. (2021). Analysis of Biofilm-Related Genes and Antifungal Susceptibility Pattern of Vaginal *Candida albicans* and Non-*Candida albicans* Species. *BioMed research international*, 2021.

Monika, S., & Zbigniew, O. (2017). Contribution of aspartic proteases in Candida virulence. Protease inhibitors against Candida infections. *Current Protein and Peptide Science*, *18*(10), 1050-1062.

Nikoomanesh, F., & Bashardoust, B. (2018). Effect of Farnesol on Responsive Gene Expressions in Hyphal Morphogenesis Transformation of Candida albicans. *Infection Epidemiology and Microbiology*, *4*(2), 73-77.

Nikoomanesh, F., Roudbarmohammadi, S., Khoobi, M., Haghighi, F., & Roudbary, M. (2019). Design and synthesis of mucoadhesive nanogel containing farnesol: investigation of the effect on *HWP1*, *SAP6* and *Rim101* genes expression of *Candida albicans in vitro*. *Artificial cells, nanomedicine, and biotechnology*, *47*(1), 64-72.

Nikou, S. A., Kichik, N., Brown, R., Ponde, N. O., Ho, J., Naglik, J. R., & Richardson, J. P. (2019). *Candida albicans* interactions with mucosal surfaces during health and disease. *Pathogens*, *8*(2), 53.

Ramage, G., Rajendran, R., Sherry, L., & Williams, C. (2012). Fungal biofilm resistance. *International journal of microbiology*, *2012*.

Shiyamalee, A., Panagoda, G. J., & Senanayake, M. R. D. M. (2020). Tomato juice as a potential replacement for human serum in germ tube test assay. *Ceylon Journal of Science*, *49*(2), 213-216.

Soliman, A. M., Abdel-Latif, W., Shehata, I. H., Fouda, A., Abdo, A. M., & Ahmed, Y. M. (2021). Green approach to overcome the resistance pattern of *Candida* spp. using biosynthesized silver nanoparticles fabricated by *Penicillium chrysogenum* F9. *Biological Trace Element Research*, *199(2)*, 800-811.

Udayalaxmi, J., & Shenoy, N. (2016). Comparison between biofilm production, phospholipase and haemolytic activity of different species of *Candida* isolated from dental caries lesions in children. *Journal of clinical and diagnostic research: JCDR*, *10(4)*, DC21.

Wongsuk, T., Pumeesat, P., & Luplertlop, N. (2016). Fungal quorum sensing molecules: role in fungal morphogenesis and pathogenicity. *Journal of basic microbiology*, *56(5)*, 440-447.

How to cite this article:

Tarek El-Said El-Banna, Fatma Ibrahim Sonbol, Sahar Nasser Al-Kharsawy and Lamiaa A. Al-Madboly. 2022. Impact of Quorum Sensing Molecules at Supra-physiological Concentrations on Virulence Factors of Multiple Drug Resistant *Candida* Clinical Isolates. *Int.J.Curr.Microbiol.App.Sci.* 11(07): 24-33.
doi: https://doi.org/10.20546/ijcmas.2022.1107.004