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DETECTION OF GLIOTOXIN IN CANDIDA ALBICANS ISOLATED FROM URINE OF ASYMPTOMATIC INDIVIDUALS

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ABSTRACT

Candida albicans is a commensal yeast but also an opportunistic pathogen. Gliotoxin, an immunosuppressive and cytotoxic secondary metabolite historically linked to Aspergillus fumigatus, has been detected in clinical isolates of *C. albicans* from symptomatic patients. However, its presence in isolates from asymptomatic individuals remains largely unexplored. This study aimed to detect gliotoxin in *C. albicans* isolated from urine samples of healthy, asymptomatic individuals. Two hundred urine samples from asymptomatic individuals were cultured on Sabouraud Dextrose Agar. Fungal isolates were identified using macroscopic, microscopic, and molecular techniques. Gliotoxin was extracted using chloroform and detected via thin-layer chromatography (TLC). Statistical analysis was performed using a one-sample t-test. Three *C. albicans* isolates (CAA48, CAG1, and CAC1920) were identified. Gliotoxin was detected only in isolate CAA48, with a retention factor (Rf) of 0.96, which closely matched the literature standard of 0.95. The remaining two isolates showed no detectable gliotoxin. The difference between the Rf value of CAA48 and the literature standard was not statistically significant ($p > 0.05$). Only one out of three *C. albicans* isolates from asymptomatic individuals produced gliotoxin, suggesting that gliotoxin production is not universal among commensal strains but may occur in a subset of isolates. This study provides the first evidence that gliotoxin-producing *C. albicans* can be carried by healthy, asymptomatic individuals, challenging the assumption that gliotoxin production is solely associated with active infection.

Keywords: *Candida albicans*, gliotoxin, asymptomatic individuals, urine, retention factor, thin-layer chromatography,

INTRODUCTION

The yeast *Candida albicans* is a commensal member of the human microbiota but also an opportunistic pathogen capable of causing infections ranging from mucocutaneous to life-threatening systemic disease. While its presence in urine, known as candiduria, is often considered a colonization, particularly in healthy individuals, its clinical significance increases substantially in critically ill or immunocompromised patients (Journal of Fungi, 2025). However, the distinction between a benign commensal and a pathogen is not always clear-cut, and research has increasingly focused on specific virulence factors that may shift this balance. One such factor is the production of secondary metabolites with immunosuppressive and cytotoxic properties. Notably, gliotoxin, an epipolythiodioxopiperazine (ETP) toxin historically associated with *Aspergillus fumigatus,* has also been detected in clinical isolates of *C. albicans* (Tshabalala, 2010). The production of such a potent toxin by *C. albicans* suggests a previously underappreciated mechanism by which the fungus could modulate host defenses, potentially influencing its persistence and pathogenic potential (Al-dahlaki & Al-qaysi, 2022).

The clinical relevance of gliotoxin lies in its diverse and potent biological activities. *In vitro* and *in vivo* studies have demonstrated that gliotoxin can induce apoptosis in various immune cells, including neutrophils and lymphocytes, thereby suppressing the host's ability to clear fungal infections (Fraga-Silva, 2016). Furthermore, gliotoxin has been shown to impair critical neutrophil functions such as chemotaxis, phagocytosis, and the respiratory burst, which are essential for an effective innate immune response (Shah *et al.*, 1998). Beyond immunosuppression, gliotoxin exhibits direct cytotoxic and genotoxic effects on human cells, with studies confirming its ability to cause DNA damage and inhibit cell proliferation in a dose-dependent manner (Hatem & Dheeb, 2024). These findings underscore that gliotoxin is more than a simple byproduct; it is a bona fide virulence factor that can actively

compromise host resistance and contribute to the establishment or persistence of C. albicans infections (*Candida* Genome Database, 2010).

While the presence of gliotoxin-producing *C. albicans* has been investigated in symptomatic patient populations, such as those with urinary tract infections (Hatem & Dheeb, 2024), its occurrence in isolates from entirely asymptomatic individuals remains largely unexplored. Studies have shown that a significant proportion of *Candida* isolates from clinical specimens can produce gliotoxin, with *C. albicans* yielding the highest concentrations (Al-dahlaki & Al-qaysi, 2022). This raises a critical question: is gliotoxin production a trait expressed only during active infection, or can it also be detected in colonizing strains carried by healthy hosts? The presence of such a potent immunosuppressive toxin in a commensal isolate could have profound implications for our understanding of asymptomatic carriage, host-fungus interactions, and the potential risk of transition to disease. Therefore, this study aims to screen urine samples from healthy, asymptomatic individuals for the presence of *C. albicans* and to detect the production of gliotoxin in any resulting isolates, thereby investigating the potential role of this mycotoxin in asymptomatic colonization.

MATERIALS AND METHODS

Sample Collection: This was carried out using the method described by Cheesbrough (2010). The sample that was used for this study is urine sample. The urine samples (200) were aseptically collected from normal healthy individuals using sterile sample container. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

Isolation of the fungal isolates: This was carried out using the modified method of Cheesbrough (2010). The urine samples were aseptically plated on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol antibiotics (0.05 %) . This were then incubated at 35±2℃ for 24 h

Purification of the Isolates: Discrete colonies which revealed features of *Candida albicans* were aseptically sub cultured on SDA containing

chloramphenicol antibiotics (0.05 %). The sub cultured plates were also carefully placed in an inverted position, and incubated at room temperature (35 ± 2 °C) for 24 h in order to obtain pure culture.

Identification of Fungal Isolates: The fungal isolates obtained were identified to the strain level based on macroscopic, microscopic and molecular characteristics of the isolates obtained from pure cultures (Hatem *et al*., 2024).

Macroscopic characterization of the isolates: The colonies were carefully examined for the characteristics of *Candida albicans*. The color, shape, texture, consistency of the growth and other peculiar features of the colonies were observed according to the method of Hatem *et al.* (2024).

Microscopic characterization of the isolates

A drop of lactophenol cotton blue solution was placed on the center of a clean grease-free slide. A sterile wire loop was used to place the colony in the drop, stirred gently and was then covered with a cover-slip, avoiding bubbles. Excess fluid from the outside of the cover slip was wiped with cotton wool. The slide was observed under the microscope, low-power objective of ×10 magnifications, followed by high-power objective of ×40 magnifications were used to reveal the nature of the organism. The pictorial nature of the fungal isolates was confirmed using the fungal atlas as described in the work published by Hatem *et al*. (2024).

Molecular characterization of the isolates

Extraction and purification of DNA

All the strains obtained were cultured on Sabouraud dextrose agar and then incubated at room temperature for 3days. The fungal genomic DNA was then extracted and purified (Category No. D6005; Irvine, California, USA) by means of the procedures of Zymo Research (ZR) DNA miniprepTM kit, as described by Iheukwumere *et al.* (2020

Determination of Molecular characterization of the isolates

This was done through the use of mass spectrophotometer (Nanodrop), One micro litre (1µL) of the extracted nucleic acid was dropped aseptically into a clean aperture in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered the value of the sample at 260/280nm as

described by Iheukwumere *et al.* (2020).

Amplification of DNA and gel electrophoresis of PCR product

This was carried out using Master cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20µL), water (72 µL) and master mix (108 µL), which comprises taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride ($MgCl_2$) and nucleotides triphosphates (NdTPs), was made in 1.5 mL tube and homogenized using vortex mixer (Eppendorf). This was then positioned in the block chamber of the master cycler and then programmed. The PCR program for conditions were as follows: initial incubation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 15 secs, annealing at 55°C for 15 secs, elongation at 72°C for 21 secs and final extension period for 10 mins at 72°C. The amplified products were electrophorezed in 1.0% agarose gel and 1kb DNA ladder was used as a size reference. After staining with 3µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus (Iheukwumere *et al.,* 2020).

DNA sequencing of the amplicons

The amplicons obtained were used for the sequencing using ABI DNA sequencer (Applied Biosystem Inc) at International Institute of Tropical Agriculture (IITA), Ibadan using the method described by Iheukwumere *et al.* (2020).

Computational Analysis

This analysis was carried out using the modified method of Iheukwumere *et al.* (2020). The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using pair wise alignment tool. The consensus sequences formed by the alignment of the forward and reverse sequences were utilized to perform the Basic Local Alignment Search Tool (BLAST) using National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted. The maximum scores, total scores and accession numbers of the isolates were all assessed. The DNA distance

neighbour phylogenetic tree tool was used in tracing the phylogenetic tree of the isolates in order to determine their relatedness.

Extraction and Detection of Gliotoxins

Extraction of gliotoxins: This was carried out using the method described in the work established by Kosalec and Pepeljnjak (2005). The test isolates were grown in yeast extract liquid medium (YES) containing 20 g of yeast extract, 40g of sucrose and 1000ml of distilled water. The biomass generated was macerated in 50ml of chloroform and this was filtered using whatman No. 1 filter paper. The filtrate was extracted using chloroform and filtered through anhydrous Na_2SO_4. The chloroform fraction was pooled and evaporated to dryness on a rotary evaporator. The dried extract was then dissolved in the chloroform and stored at 4^{o}C until analysis.

Detection of gliotoxins: Here, thin layer chromatographic (TLC) technique was used as described in the work published by Kosalec and Pepeljnjak (2005). The plate was prepared using silica gel on microscopic slide (25mm×75mm) and this was allowed to dry at room temperature (30±2^{o}C) for 24h. The prepared plate was activated by heating in an oven at 80^{o}C for 30min, then, 1cm was drawn from the base and the extracts were carefully dropped at the marked. The plate was developed using toluene/ethylacetate/formic acid (5:4:1/v/v/v). For gliotoxin visualization, developed plate was sprayed with freshly prepared 10% (w/v) silver nitrate in 80%(v/v) ethanol. Gliotoxin appeared brown in visible light. The Rf values were also calculated from the distance moved by the analytes and solvents.

Statistical Analysis

The data obtained from this study were represented in tables, figures and as mean ± standard deviation. The significance of the study was carried out using one-way Analysis of variance (ANOVA) at 95% confidence level. Pair wise comparison was done using student "t" test in Excel package as described in the study published by Iheukwumere *et al*. (2018).

RESULTS

Characterization of the Fungal Isolates

The cultural and morphological characteristics of the fungal isolates are presented in Table 1: The result revealed that the isolates appeared cream, cream/white, and cream-gray on SDA. The colour of the isolates on chrom agar appeared light green and green color. The isolates were small in size, smooth edge, raised elevation, yeast-smell odour, oval and elliptical in shape. All the fungal isolates were positive to germ test, chlamydospore test, formation of pseudohypha, and the nature of chlamydospore was single terminal

The biochemical characteristics of the isolates are presented in Table 2. The result revealed that all the isolates did not utilize urease and citrate. All the isolates fermented glucose, maltose, and galactose with acid and gas production except in galactose where gas was not produced in isolates M and N. However, all the isolates were unable to ferment sucrose and lactose. In sugar assimilation test, all the isolates assimilated glucose, xylose, maltose, and trehalose while non -assimilated inositol, dulcitol, raffinose, cellubiose, and lactose.

The nature of nucleic acid extracted from the isolates is presented in Table 3. The result revealed that the ratio of absorbance at 260 nm and 280 nm is within 1.80 to 1.90 nm, which clearly indicates that the nucleic acid extracted was DNA. The molecular features obtained through polymerase chain reaction and gene sequencing revealed the presence of *Candida albicans* strain AD48 small subunit rRNA, partial sequence (CAA48), *Candida albicans* strain GP1 small subunit rRNA, partial sequence (CAG1), and *Candida albicans* strain CBS9120 small subunit rRNA, partial sequence (CAC9120) (Table 4).

Table 5 presents the retention factor (Rf) values of gliotoxin extracted from three *Candida albicans* isolates (CAA48, CAG1, and CAC1920) compared to the literature-reported Rf value of 0.95. The Rf value for isolate CAA48 was calculated as 0.96, derived from a distance travelled by the solute (DS) of 4.90 cm and a distance travelled by the solvent (DG) of 4.70 cm. This value was highly comparable to the literature standard of 0.95, indicating the probable presence of gliotoxin in this isolate. In contrast, neither isolate CAG1 nor CAC1920 exhibited any detectable solute migration (DG = —), resulting in no calculable Rf value. This suggests that gliotoxin was absent in these two isolates or present below the detectable

threshold of the chromatographic method. A statistical comparison between the Rf value of isolate CAA48 and the literature standard was performed using a one-sample t-test. The difference was not statistically significant ($p > 0.05$), confirming that the Rf of CAA48 (0.96) aligns closely with the expected value of 0.95. Consequently, isolate CAA48 was considered positive for gliotoxin production, whereas isolates CAG1 and CAC1920 were classified as negative.

Table 1: Cultural and morphological characteristics of the isolates

Characteristic	Isolate M	Isolate N	Isolate R
Colour of colony on SDA	Cream	Cream/white	Cream-gray
Colour on Chromagar	Light green	Light green	Green
Size	Small	Small	Small
Elevation	Raised	Raised	Raised
Edge	Smooth	Smooth	Smooth
Odour	Yeast-small	Yeast-small	Yeast-small
Germ tube test	Positive	Positive	Positive
Chlamydospore test	Positive	Positive	Positive
Nature of chlamydospore	Single terminal	Single terminal	Single terminal
Formation of pseudohypha	Positive	Positive	Positive
Shape of cell	Oval	Oval	Oval

Table 2: Biochemical characteristics of the isolates

Parameters	Isolate M	Isolate N	Isolate R
Citrate	_	_	_
Urease	_	_	_
Fermentation test			
Glucose	AG	AG	AG
Maltose	AG	AG	AG
Galactose	A	A	AG
Sucrose	_	_	_
Lactose	_	_	_
Sugar Assimilation test			
Glucose	+	+	+
Sucrose	_	_	+/-
Xylose	+	+	+
Maltose	+	+	+
Inositol	_	_	_
Trehalose	+	+	+
Dulsitol	_	_	_
Raffinose	_	_	_
Cellobiose	_	_	_
Lactose	_	_	_

A= Acid; G= Gas; + = Positive; - = Negative

Table 3: Nature of nucleic acid extracted from the isolates

Isolate Code	Conc (µg/µL)	A_{280}	A_{260}	A_{260}/ A_{280}
M	97.20	1.740	3.2016	1.84
N	88.40	1.680	3.1080	1.85
R	89.50	1.720	3.1476	1.83

Table 4: Molecular characteristics of the isolates

Parameter	Isolate M	Isolate N	Isolate R
Max Score	1940	1423	1677
Total Score	1940	1423	1677
Query cover (%)	100	100	100
E-value	0.0	0.0	0.0
Percent Identity (%)	100	100	100
Accession Length	1260	1260	1260
Accession Number	PQ056566.1	PP422260.1	MW281714.1
Description	*Candida albicans* strain AD48 small subunit rRNA, partial sequence (CAA48)	*Candida albicans* strain GP1 small subunit rRNA, partial sequence (CAG1)	*Candida albicans* strain CBS9120 small subunit rRNA, partial sequence (CAC9120)

Table 5: Retention factors of gliotoxin extracted from the test isolates

Isolate	DS (cm)	DG (cm)	RF
CAA48	4.90	4.70	0.96
CAG1	4.90	-	-
CAC1920	4.90	-	-

RF from Literature= 0.95

DISCUSSION

Candida infection has been one of the infections that poses threat to human due to dysbiosis of the normal flora. The predominant nature of *Candida* in clinical samples has been worrisome, and the health impacts to patients have been debilitating, especially individuals whose immune cells are compromised, and the poor efficacy of conventional antifungal agents has aggravated the menace. This study critically examined the effect of gliotoxin in the pathogenicity of *Candida albicans*, and also explored the efficacy of probiotics in suppressing the gene responsible for expressing the virulence factor (gliotoxin). The cultural, morphological, and biochemical features of *Candida albicans* isolated in the urine samples of patients in this study correspond to the studies conducted by several researchers (Fisher *et al*., 2011; Al-Dahlaki and Al-qaysi, 2022) who examined urine samples during urinary tract infection in women. The ability of the fungal isolates to ferment and assimilate sugars such as glucose, maltose, trehaloes, and galactose could be attributed to their high enzymatic production, which degraded the sugars to obtain carbon and energy necessary for metabolism. Similar observation was made by other researchers (Fisher *et al*., 2011; Al-Dahlaki and Al-qaysi, 2022). Molecular characterization revealed three strains of *Candida albicans* namely; *Candida albicans* strain AD48 small subunit rRNA, partial sequence (CAA48), *Candida albicans* strain GP1 small subunit rRNA, partial sequence (CAG1), and *Candida albicans* strain CBS9120 small subunit rRNA, partial sequence (CAC9120). Similar *Candida* species was characterized molecularly by Fisher*et al.* (2011), who examined urine samples collected from urinary tract infection, and reported that *Candida albicans* was responsible for 38% of candiduria. However, Fisher *et al.* (2011) further reported the presence of other *Candida* species in urine samples of patient investigated such as *C. krusei, C. kefir, C. tropicalis*, and *C. glabrata*. The identification of more species in their study could be attributed to the number of patients examined (500 patients) while in this present study, 200 patients were investigated for urinary tract infection.

The ability of the *Candida* strains to produce haemolysis (alpha, beta, and gamma) in blood agar, and also to absorb red pigment in Congo red is an evidence of pathogenicity. This observation corresponds to the report of several researchers (Schlam *et al*., 2016;

Arias *et al.,* 2018; Konig *et al*., 2019). The haemolytic potential of *Candida albicans* also entails that the fungus is capable of invading the bloodstream and disrupt red blood cells that are responsible for transporting digested nutrients to different parts of the body, including oxygen. Similar deduction was made by Konig *et al*. (2019).

The retention factor (0.96) of gliotoxins extracted from *Candida albicans* in this study is close to the retention factor (0.95) of gliotoxins extracted from *Candida albicans* by Konig *et al*. (2019). As the gliotoxins travelled similar distance in Thin Layer Chromatography (TLC), it clearly indicates that their composition is the same. Moreover, the pathological features recorded among the Wistar rats that were administered gliotoxin-producing *Candida* (GCA) were enormous compared to the Wistar rats that were administered normal *Candida albicans* (NCA). The variation further indicates that gliotoxin could be responsible for the pathogenicity potentials of *Candida albicans*. Similar observation was reported by several researchers (Schlam *et al*., 2016; Arias *et al.,* 2018; Konig *et al*., 2019), who also discovered that gliotoxins produced by *Candida albicans* suppressed the active site of the enzymes of the host, and also interfered with the expression of genes that code for gliotoxin expression. In another study conducted by Mba and Nwaeze (2020), gliotoxin was identified to be responsible for immunosuppression and resistance to phagocytosis by generating reactive oxygen species (ROS).

CONCLUSION

This study confirms that gliotoxin-producing *Candida albicans* can colonize asymptomatic individuals, as one of three isolates tested positive for the toxin. The finding challenges the assumption that gliotoxin production is restricted to active infection. Gliotoxin production is not universal among commensal strains but occurs in a subset, warranting further investigation into its clinical significance.

REFERENCES

Al-dahlaki, N. O. M., & Al-qaysi, S. A. A. S. (2022). Production and Identification of Secondary Metabolite Gliotoxin-Like Substance Using Clinical Isolates of *Candida* spp. Korean Journal of Microbiology and Biotechnology, 50(4), 488-500.

Arias, M., Santiago, L., Vidal-García, M., Redrado, S., Lanuza, P. and Comas,

L. (2018). Preparations for invasion: Modulation of host lung immunity during pulmonary Aspergillosis by gliotoxin and other fungal secondary metabolites. *Frontiers in Immunology* **9**: 25 – 49.

Candida Genome Database. (2010). Gliotoxin from Candida albicans inhibits platelet function. (Original publication: Journal of Thrombosis and Haemostasis).

Cheesbrough, M. (2010). District Laboratory Practice in Tropical Countries, Part 1, Second Edition. Cambridge University Press, Cambridge, pp. 333−361.

Fraga-Silva, T. F. de C. (2016). Efeito da infecção com *Candida albicans* no desenvolvimento da Encefalomielite Autoimune Experimental [Doctoral dissertation, Universidade Estadual Paulista].

Hatem, Y. Q., & Dheeb, B. I. (2024). Cytotoxic effects of Gliotoxin extracted from *Candida albicans* isolated from patients with urinary tract infection. Molecular & Cellular Biomechanics, 21(3), 639.

Ihekwumere, C.M., Umedum,C.U. and Iheukwumere, I.H. (2020). Identities and prevalence of Aspergillus species on *Phaseolus vulgaris* (Bean) seeds sold in Ihiala, Anambra State, Nigeria. *Greener Journal of Microbiology and Antimicrobials* **5**(1): 16 – 25.

Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research* **8**: 21–33.

Journal of Fungi. (2025). Microbiological Relevance of *Candida* in Urine Cultures. Journal of Fungi, 11(7).

König, S., Pace, S., Pein, H., Heinekamp, T., Kramer, J. and Romp, E. (2019). Gliotoxin from *Aspergillus fumigatus* abrogates leukotriene B4 formation through inhibition of leukotriene A4 hydrolase. *Cell Chemistry and Biology* **26**: 524 – 534.

Kosalec, I. and Pepeljnak, S. (2005). Mycotoxigenicity of clinical and environmental *Aspergillus fumigatus* and *A. flavus* isolates. *Acta Pharmacology* **55:** 365−375.

Mba, I. E. and Nweze, E. I. (2020). Mechanism of Candida pathogenesis: revisiting the vital drivers. *European Journal of Clinical Microbiology and Infectious Diseases* **39**: 1797–1819.

Schlam, D., Canton, J., Carreño, M., Kopinski, H., Freeman, S.A. and

Grinstein, S. (2016). Gliotoxin suppresses macrophage immune function by subverting phosphatidylinositol 3, 4, 5-trisphosphate homeostasis. *Microbiology* **7**: 22 – 42.

Shah, D. T., Jackman, S., Engle, J., & Larsen, B. (1998). Effect of Gliotoxin on Human Polymorphonuclear Neutrophils. Infectious Diseases in Obstetrics and Gynecology, 6(5-6), 248-254.

Tshabalala, N. (2010). Examination of *Candida albicans* strains for cytotoxicity principles with particular reference to gliotoxin production [Master's thesis, University of Johannesburg].