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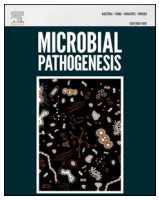

Extracellular hydrolytic enzyme activities and biofilm formation in *Candida* species isolated from people living with human immunodeficiency virus with oropharyngeal candidiasis at HIV/AIDS clinics in Uganda

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A R T I C L E I N F O

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A B S T R A C T

Background: Commensal oral *Candida* species can become opportunistic and transition to pathogenic causes of oropharyngeal candidiasis (OPC) in individuals with impaired immunity through ecological cues and the expression of extracellular hydrolytic enzyme activities and biofilm formation.
Objective: We evaluated phospholipase, proteinase, hemolysin, esterase, and coagulase enzymatic activities and biofilm formation in *Candida* species isolated from people living with human immunodeficiency virus (PLHIV) with OPC.
Methods: Thirty-five *Candida* isolates from PLHIV with OPC were retrieved from a sample repository and evaluated for phospholipase activity using the egg yolk agar method, proteinase activity using the bovine serum albumin agar method, hemolysin activity using the blood agar plate method, esterase activity using the Tween 80 opacity test medium method, coagulase activity using the classical tube method, and biofilm formation using the microtiter plate assay method *in vitro*.
Results: A total of 35 *Candida* isolates obtained from PLHIV with OPC were included in this study, and phospholipase and proteinase activities were detected in 33/35 (94.3 %) and 31/35 (88.6 %) *Candida* isolates, respectively. Up to 25/35 (71.4 %) of the *Candida* isolates exhibited biofilm formation, whereas esterase activity was demonstrated in 23/35 (65.7 %) of the *Candida* isolates. Fewer isolates (21/35, 60 %) produced hemolysin, and coagulase production was the least common virulence activity detected in 18/35 (51.4 %) of the *Candida* isolates.
Conclusion: Phospholipase and proteinase activities were the strongest in oropharyngeal *Candida* species.

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1. Introduction

Approximately 38 million individuals are living with the human immunodeficiency virus (HIV) globally, with approximately 68 % (25.7 million) of these people residing in Africa [1]. Oropharyngeal candidiasis (OPC) is the most prevalent opportunistic superficial fungal infection caused among people living with human immunodeficiency virus (PLHIV) [2]. It occurs in approximately 90 % of PLHIV when CD4 T-cell counts drop below 200 cells/μL [3–5]. Varying prevalence rates of OPC among PLHIV have been reported in African countries, for example, 43%–73 % in Cameroon [6–8], 79 % in South Africa [9], 4.9%–60 % in Nigeria [10–13], 75.3 % in Ghana [14], 79.4 % in the Ivory Coast [15], and 7.6%–52 % in Uganda [16,17]. The burden of OPC among PLHIV in Uganda is a concern, as severe forms can lead to swallowing difficulties, reduced food intake, oral cancer, and impaired quality of life [18].

While *Candida albicans* remains the most common cause of OPC, accounting for 48 %–87 % of cases [19], there has been a reported shift towards non-*albicans Candida* (NAC) species [7,16]. In addition to reduced host immunity and ecological clues, *Candida* species secrete various extracellular hydrolytic enzymes and form biofilms as virulence factors for pathogenesis during the OPC infection process [20]. For example, phospholipases disrupt host cell phospholipids, leading to host cell lysis and tissue invasion, whereas proteinases interrupt surface proteins and defense mechanisms, leading to tissue invasion [20]. Whereas hemolysin lyses host erythrocytes and obtains iron for metabolism, facilitating host tissue invasion, esterases have the ability to hydrolyze ester bonds, enhancing binding to host cells, penetration, and invasion [21]. Coagulase binds to fibrinogen and activates prothrombin to convert fibrinogen to fibrin, leading to fibrin clots, which protect *Candida* species from being phagocytosed by granulocytes [22]. Furthermore, biofilm production and maintenance within biofilms protect *Candida* against the environment, antifungal drugs, host immune defense, and chemical and physical stresses, leading to antifungal treatment failure and the progression of OPC [23]. This has made extracellular hydrolytic enzymes and biofilm production among *Candida* species vital virulent markers that potentiate OPC causation [24]. Additionally, immunological status, such as low $CD4^+$ T-cell counts and high viral load counts, has been cited as an immunological predictor for OPC among PLHIV [16]. Recent studies have isolated *C. albicans* and NAC species such as *Candida parapsilosis, Candida glabrata, Candida tropicalis, Candia dubliniensis*, *Candia krusei, Candida lusitaniae, Candida guilliermondi*, and *Candida norvegensis* from the oral cavity of PLHIV with OPC in Uganda [16,25]. However, the question of their extracellular hydrolytic enzyme activities and biofilm formation has long been unanswered. Therefore, we evaluated phospholipase, proteinase, hemolysin, esterase and coagulase activities and biofilm formation in *Candida* species isolated from PLHIV with OPC on antiretroviral therapy (ART) at AIDS Support Organization (TASO) clinics in Uganda.

2. Materials and methods

2.1. Study design and setting

This was a cross-sectional study involving 29 PLHIV with OPC on ART at the TASO Mulago and Mbarara regional referral clinics in central and southwestern Uganda, respectively. A total of 35 oropharyngeal *Candida* isolates from these 29 participants were retrieved from the sample repository of our previous study [26] for *in vitro* evaluation of extracellular hydrolytic enzyme activities and biofilm formation.

2.2. Sample size calculation

As detailed in our previous research [26], we employed a standard formula by Kish and Leslie for sample size calculation, which resulted in a total of 384 PLHIV as our study participants on the basis of a 52 % prevalence of OPC among PLHIV in Southwest Uganda [27].

This formula by Kish and Leslie is as follows:

$$N = \frac{Z^2 P(1-P)}{d^2}$$

N = study sample size required
Z = critical value associated with 95 % confidence interval = 1.96
P = estimated proportion of PLHIV with OPC
d = margin of error = 0.05

$$N = \frac{1.96^2 \times 0.52 \times (1-0.52)}{0.05^2} = 384$$

This calculation resulted in a sample size of 384 participants. From this cohort of 384 PLHIV, we successfully isolated 35 *Candida* strains from 29 individuals, which corresponded to a prevalence of 7.6 % for OPC among PLHIV [26]. The primary objective of this nested study was to investigate the activities of extracellular hydrolytic enzymes and the formation of biofilms in these 35 specific *Candida* isolates from 29 PLHIV with OPC.

2.3. Preparation of retrieved isolates for studies

Candida isolates that were preserved in brain heart infusion (BHI) broth supplemented with 10 % glycerol at −80 °C and BHI were retrieved from a sample repository freezer. The isolates were thawed by gently warming them to room temperature for 1 h.

2.4. Isolation and identification of Candida species

A loopful of the culture was streaked onto Sabouraud dextrose agar (Oxoid, Basingstoke, UK) supplemented with 50 μg/1 ml gentamicin and then incubated at 32 °C for 48–72 h aerobically to isolate *Candida* species. For rapid identification, a matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) BioTyper 4.1 system (Bruker Daltonics) at the Department of Microbiology, Makerere University, was used to identify *Candida* species as previously described by Bader and colleagues [28].

2.5. Enzyme activities and biofilm formation assays

The *Candida* isolates were suspended in sterile phosphate-buffered saline (PBS) and matched to 2 McFarland to carry out the virulence assays.

To assess the phospholipase, proteinase, hemolysin, esterase, and coagulase activities and biofilm formation in *Candida* isolates obtained from PLHIV with OPC, each isolate was tested in duplicate in each assay, and three assays were carried out for each isolate on separate occasions. The mean of the six values obtained was considered for analysis.

2.6. Determination of phospholipase activity

The phospholipase activity (Pz) was analyzed using the egg yolk agar method [29,30]. Approximately 10 μl of a standardized yeast suspension (10^8 CFU/ml) was pipetted, spotted onto fresh egg yolk agar plates, and left to dry in a biosafety cabinet. The culture was then incubated at 37 °C aerobically for 48 h, after which the diameter of the precipitation zone around the colony was determined. Pz was measured by dividing the diameter of the colony (Cd) by the total diameter of the colony plus the precipitation zone (Pd). *C. albicans* ATCC 10231 was chosen as a positive control, whereas *Candida kefyr* 2512 was used as a negative control [31]. All isolates were tested in duplicate on three separate occasions, and the obtained mean of the six values was considered for analysis.

$$\text{Pz value} = \frac{Cd}{Cd + Pd}$$

Pz activity was scored as negative when Pz = 1, weak when Pz =

0.64–0.99, and strong when Pz ≤ 0.63 [24,32], indicating that the lower Pz was, the greater the phospholipase activity (Fig. 1A)

2.7. Determination of proteinase activity

Proteinase activity was determined using the bovine serum albumin agar (BSA) method [33]. Using a 24-h-old culture, a yeast suspension of approximately 1 × 10^8 CFU/ml was prepared with 1 ml of 0.85 % normal saline and a turbidometer. Ten microliters of the standardized yeast suspension were pipetted and spotted onto sterile BSA agar plates. The inoculated plates were then incubated at 37 °C for 5 days under aerobic conditions. After incubation, the plates were fixed with 20 % trichloroacetic acid and stained with 0.25 % w/v Coomassie blue. Decolorization was performed by flooding the culture plates with 15 % acetic acid. Proteinase activity was detected by the presence of a clear halo around the yeast colonies. The diameter of the halo clearance (Hd) zone relative to the diameter of the colonies (Cd) was used to assess the degree of proteinase activity (Prz) [30]. *C. albicans* ATCC 10231 was used as a positive *control, and C. kefyr* 2512 was used as a negative control for this experiment.

$$\text{Prz value} = \frac{\text{Cd}}{\text{Cd} + \text{Hd}}$$

Prz activity was scored as negative when the Prz value = 1, weak when Prz = 0.64–0.99, and strong when Prz ≤0.63 [24,32], meaning that a low Prz value indicated strong production of the proteinase enzyme (Fig. 1B).

2.8. Determination of hemolysin activity

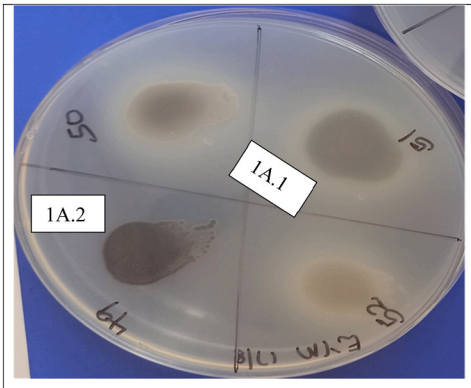
The hemolytic activity of *Candida* species was determined by the blood agar plate method [34]. Ten (10) microliters of standardized yeast suspension (10^8) were inoculated onto blood agar plates, which were then incubated at 37 °C in 5 % carbon dioxide for 48 h. After incubation, a transparent/semitransparent zone around the inoculation site was considered to indicate positive hemolytic activity [34]. The ratio of the diameter of the colony (Cd) to that of the translucent zone of hemolysis (Hd) (mm) was used as the hemolytic index (Hz value). *C. albicans* ATCC 90028 was used as a positive control, and *C. parapsilosis* ATCC 2201 was used as a negative control. All isolates were tested in duplicate on three separate occasions, and the obtained mean of the six values was considered for analysis.

$$\text{Hz value} = \frac{Cd}{Hd}$$

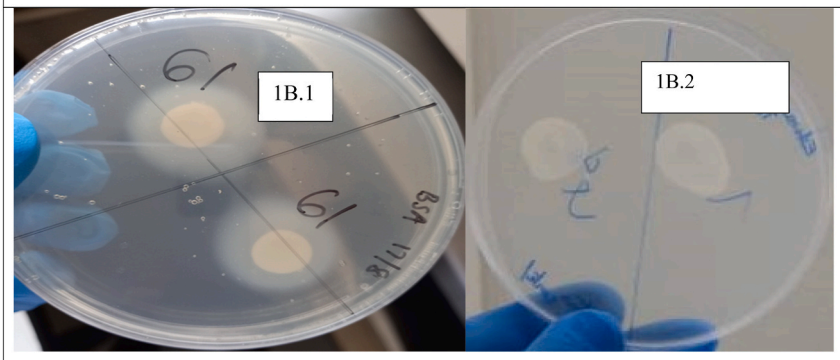
Hz activity was scored as negative when the Hz value = 1, weak when Hz = 0.64–0.99, and strong when Hz ≤ 0.63 [24,32], meaning that a low Hz value indicated strong hemolytic activity.

2.9. Determination of esterase activity

The esterase activity of *Candida* species was detected using the



A: Photograph showing phospholipase activity of the candida isolate inoculum by egg yolk agar method. Ten microliters of Candida culture suspension were spotted on egg yolk agar plate. The plate was observed and diameter of the precipitation was observed after 48 hours of incubation. A distinctive precipitation around the inoculum site was shown in 1A.1, which shown a positive phospholipase activity. No precipitation could be seen in 1A.2 and therefore considered no phospholipase activity



B: Photograph showing proteinase activity of the candida isolate inoculum on bovine serum albumin plate. Ten microliters of Candida culture suspension were spotted on bovine serum albumin plate. The plate was observed and diameter of the halo was observed after 48 hours of incubation. A distinctive translucent halo around the inoculum site was shown in 1B.1, which shown a positive proteinase activity. No halo could be seen in 1B.2 and therefore considered no proteinase activity

Fig. 1. Depicting the phospholipase and proteinase hydrolytic enzyme activities of the Candida isolates.

Tween 80 opacity test medium method [35]. Ten microliters of the previously prepared suspension were carefully spotted on Tween 80 opacity test medium and left to dry. The inoculated agar plates were incubated at 30 °C and examined daily for 10 days [35]. The presence of a halo around an inoculated site on tween 80 opacity test medium indicated positive esterase activity [36]. Esterase activity (Ez) was determined as the ratio of the diameter of the colonies (Cd) to the diameter of the clear halo with calcium precipitates around the colony (Hd) [24]. *C. albicans* ATCC10231 served as a positive control for this experiment. All isolates were tested in duplicate on three separate occasions, and the obtained mean of the six values was considered for analysis.

$$\text{Ez value} = \frac{Cd}{Hd}$$

Ez activity was considered negative when Ez = 1, weak when Ez = 0.64–0.99, and strong when Ez ≤ 0.63 [24]. Thus, a low Ez value indicates strong esterase production.

2.10. Determination of coagulase activity

Coagulase activity was determined by the classical tube coagulase method [37]. Briefly, *Candida* isolates were first standardized to match 2 McFarland's turbidity standard, and 0.5 ml of this standardized cell suspension was added to 0.5 ml of 10 % rabbit plasma in a tube. The inoculated tubes were then incubated at 37 °C and observed for clot formation after 4 h. The presence of a visible clot that could not be resuspended by gentle shaking was scored 1 for coagulase-positive. Test tubes without clots were re incubated at 37 °C and reexamined after 24 h. The absence of a visible clot scored 0 for coagulase-negative. *Staphylococcus aureus* ATCC 25923 was used as a positive control, whereas *Staphylococcus epidermidis* ATCC 14990 served as a negative control. Each isolate was tested in duplicate on three separate occasions, and the obtained mean of the six values was considered for analysis.

2.11. Determination of biofilm formation

The ability of *Candida* isolates to form biofilms was analyzed using the microtiter plate assay (Mtp) method [38]. Fresh 24-h broth from *Candida* cultures was used for this assay. The cultures were grown in yeast peptone dextrose (YPD) broth, and the cell suspension was then adjusted to 2 McFarland's standard using fresh YPD broth. This suspension was further diluted 20-fold to a concentration of approximately 5×10^6 CFU/ml. Then, 180 μl of sterile YPD broth was aseptically transferred to sterile 96-well polystyrene microtiter plates. Then, 20 μl of the standardized yeast suspensions were added to each well containing 180 μl of YPD for a final concentration of approximately 5×10^5 CFU/ml. The microtiter plates were then incubated at 37 °C for 48 h. At the end of the incubation period, excess planktonic cells and broth were removed, and the plates were washed with phosphate-buffered saline three times to remove unattached cells and media components. The plates were blotted with blotting paper, inverted, and left to dry. The sessile cells from which biofilms formed were then fixed by adding 150 μl of methanol to the wells for 20 min. The methanol was then removed by inverting the plates, and the liquid was removed. The plates were then washed with phosphate-buffered saline and dried off with blotting paper. Two hundred microliters of 1 % crystal violet were added, and the mixture was left to stand for 15 min at 37 °C. At the end of the incubation period, crystal violet was added, and the plates were washed 3 times with Dulbecco's phosphate-buffered saline and left to air dry. After air drying, the stained biofilms were resolubilized using 150 μl of 33 % glacial acetic acid, and the plates were carefully agitated using a rotatory shaker. The optical density of the microtiter plates was measured spectrophotometrically at 620 nm with a spectrophotometer at 600 nm. *C. albicans* ATCC 10231 was used as a positive control, while uninoculated wells that contained sterile YPD were used as negative controls and treated as blanks. The blank absorbance values (ODc) were used to determine whether biofilms were formed by the isolates. The wells containing isolates whose optical density (OD) values were greater than those of the blank well were considered biofilm producers. All isolates were tested in duplicate on three separate occasions, and the obtained mean of the six values was considered for analysis.

The OD values were used to calculate the cutoff values (ODc) of the isolates for biofilm formation categorization and interpretation as previously described by Kırmusaoglu et al. [38] as follows: OD ≤ ODc = no biofilm formation, ODc < OD ≤ 2ODc = weak biofilm formation, 2ODc < OD ≤ 4ODc = moderate biofilm formation, 4ODc < OD = strong biofilm formation (Fig. 2 and Table 3)

2.12. Data analysis

Statistical analyses were performed using Stata version 17.0 software. Descriptive statistics, proportions, and means were used to summarize the distributions of different virulence attributes. The chi-square test was used to test the associations between biofilm formation and coagulase activity in *C. albicans* and NAC. The independent student's *t*-test was used to determine the mean differences in phospholipase, proteinase, hemolysin, and esterase activities between NAC and *C. albicans,* with a P value < 0.05 indicating statistical significance.

3. Results

We evaluated phospholipase, proteinase, hemolysin, esterase, and coagulase activities and biofilm formation in 35 *Candida* isolates from 29 participants. Among the 35 isolates, 20 were *C. albicans*, *C. tropicalis* (n = 4), *C. glabrata* (n = 4), *C. parapsilosis* (n = 2), *C. dubliniensis* (n = 2), *C. krusei* (n = 2), and *C. lusitaniae* (n = 1).

3.1. Demographic and immunological profiles of the study participants

A total of 29 PLHIV with OPC were enrolled, 22 (75.9 %) and 7 (24.1 %) from the Mulago and Mbarara TASO clinics, respectively. Overall, there were 8 males (27.6 %) compared with 22 females (72.4 %), with a mean age of 45 years (SD = 11.8). The majority of participants were single (55.2 %) and had secondary education (55.2 %). Most participants (24, 82.8 %) had a viral load >1000 copies/ml, and 14 (48.3 %) had a CD4 count ≤200 cells/μL (Table 1).

Phospholipase (Pz) activity was detected in 33/35 (94.3 %) of the total isolates and in 20/20 (100 %) and 13/15 (86.7 %) of the *C. albicans* and NAC isolates, respectively. In terms of specific NAC species, all 4/4 (100 %) *C. glabrata*, 2/2 (100 %) *C. parapsilosis*, 4/4 (100 %) *C. tropicalis* and 2/2 (100 %) *C. krusei* strains and 1/2 (50 %) *C. dubliniensis* strains

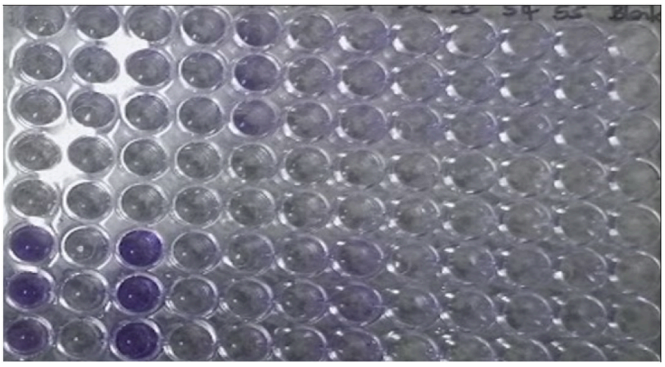


Fig. 2. Images depicting the biofilm formation by the Candida isolates in a 96-well crystal violet stained microtiter plate. Variability in biofilm formation was noted in a 96-well microtiter plate that had been indicated by the measurement of microtiter plate optical density. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Demographic and immunological profile of people living with human immunodeficiency virus with oropharyngeal candidiasis.

Profile		Overall (29) N (%)
Demographic		
Study site	TASO Mulago	22(75.9)
	TASO Mbarara	07(24.1)
Gender	Male	08 (27.6)
	Female	21 (72.4)
Age (in years)	Mean (SD)	45 (11.8)
Age group	18–29	03 (10.3)
	30–39	08 (27.6)
	40–49	08 (27.6)
	50–59	04 (13.8)
	60 - and above	06 (20.7)
Level of Education	No formal education	00 (0.0)
	Primary	12 (41.4)
	Secondary	16 (55.2)
	Tertiary	04 (13.8)
Marital Status	Single	16 (55.2)
	Married	06 (20.7)
	Divorced	02 (6.9)
	Cohabiting	03 (10.3)
	Widow	02 (6.9)
Immunological		
CD4 Count in cells/μL	≤200	14 (48.3)
	>200	15 (51.7)
Viral load in copies/ml	≤1000	5(17.2)
	>1000	24 (82.8)

SD: Standard deviation.

exhibited Pz activity. Furthermore, 14/20 (70 %) *C. albicans*, 2/4 (50 %) *C. tropicalis* and 1/2 (50 %) *C. krusei* strains presented strong Pz activity (Table 2). Additionally, the Pz values ranged from 0.39 to 0.94 for *C. albicans* and from 0.38 to 0.79 for the NAC isolates, and no significant difference was noted in the mean Pz values of the *C. albicans* (0.58 ± 0.22) and NAC isolates (0.57 ± 0.13; $p = 0.88$) (Fig. 3).

Proteinase (Prz) activity was noted in 31/35 (88.6 %) of the total isolates and in 19/20 (95 %) and 12/15 (80 %) of the *C. albicans* and NAC isolates, respectively. All 4/4 (100 %) *C. glabrata*, 4/4 (100 %) *C tropicalis*, 2/2 (100 %) *C. krusei*, 1/2 (50 %) *C. dubliniensis* and 1/2 (50 %) *C. parapsilosis* isolates exhibited Prz activity (Table 2). In general, 13/20 (65 %) *C. albicans*, 1/2 (50 %) *C. dubliniensis,* and 2/4 (50 %) *C. glabrata* strains presented strong Prz activity. Furthermore, the Prz values ranged from 0.43 to 0.92 for the *C. albicans* isolates and from 0.4 to 0.83 for the NAC isolates. However, there was no significant difference in the mean Prz values of the *C. albicans* (0.65 ± 0.19) and NAC isolates (0.59 ± 0.16; $p = 0.37$) (Fig. 3).

Hemolytic (Hz) activity was noted in 21/35 (60 %) of the total isolates, 12/20 (60 %) of the *C. albicans* isolates and 9/15 (60 %) of the NAC isolates. All 4/4 (100 %) *C. glabrata*, 2/2 (100 %) *C. krusei*, and 3/4 (75 %) of the *C tropicalis* isolates exhibited Hz activity. Additionally, 10/20 (50 %) *C. albicans* strains and 1/4 (25 %) *C tropicalis* strains presented strong Hz activity (Table 2). The Hz values ranged from 0.43 to 0.93 for the *C. albicans* isolates and from 0.46 to 0.93 for the NAC isolates. No significant difference was noted in the mean Hz values of the *C. albicans* (0.72 ± 0.2) and NAC isolates (0.68 ± 0.19; $p = 0.65$) (Fig. 3).

Esterase (Ez) activity was detected in 23/35 (65.7 %) of the total isolates, 16/20 (80 %) of the *C. albicans* isolates and 7/15 (46.7 %) of the NAC isolates. Furthermore, 3/4 (75 %) of the *C. glabrata* isolates, 3/4 (75 %) of the *C tropicalis isolates*, and 1/2 (50 %) of the *C. krusei* isolates exhibited Ez activity. Furthermore, 1/2 (50 %) *C. krusei*, 7/20 (35 %) *C. albicans,* 1/4 (25 %) *C tropicalis* and 1/4 (25 %) *C. glabrata* strains presented strong Ez activity (Table 2). The Ez values ranged from 0.35 to 0.83 for the *C. albicans* isolates and from 0.56 to 0.93 for the NAC isolates. A significant difference was noted in the mean Ez values of the *C. albicans* (0.57 ± 0.17) and NAC isolates (0.70 ± 0.14; $p = 0.04$) (Fig. 3).

Coagulase activity was observed in 18/35 (51.4 %) of the *Candida* isolates and in 13/20 (65 %) and 5/15 (33.3 %) of the *C. albicans* and NAC isolates, respectively. Among the specific NAC isolates, 1/2 (50 %) of the *C. dubiniensis* isolates, 1/2 (50 %) of the *C. glabrata* isolates and 1/2 (50 %) of the *C tropicalis* isolates had coagulase activity, whereas all the *C. lusitaniae, C. parapsilosis* and *C. krusei isolates* had no coagulase activity (Table 2).

Table 2
Extracellular hydrolytic enzymatic activity and biofilm formation by different *Candida* species.

Virulence	Score	Activity Index	*C. albicans* (20) n (%)	*Non albicans Candida* (15)						Total
				C. dubliniensis (2) n (%)	C. glabrata (4) n (%)	C. lusitaniae (1) n (%)	C. parapsilosis (2) n (%)	C. tropicalis (4) n (%)	C. krusei (2) n (%)	35 n(%)
Phospholipase	Strong	≤0.63	14(70)	0(00)	1(25)	0(00)	0(00)	2(50)	1(50)	18(51.4)
	Weak	0.64–0.99	6(30)	1(50)	3(75)	0(00)	2(100)	2(50)	1(50)	15(42.9)
	Negative	1	0(0)	1(50)	0(00)	1(100)	0(00)	0(00)	0(00)	2(5.7)
Proteinase	Strong	≤0.63	13(65)	1(50)	2(50)	0(00)	0(00)	1(25)	0(00)	17(48.6)
	Weak	0.64–0.99	6(30)	0(00)	2(50)	0(00)	1(50)	3(75)	2(100)	14(40.0)
	Negative	1	1(5)	1(50)	0(00)	1(100)	1(50)	0(00)	0(00)	4(11.4)
Hemolysin	Strong	≤0.63	10(50)	0(00)	0(00)	0(00)	0(00)	1(25)	0(00)	11(31.4)
	Weak	0.64–0.99	2(10)	0(00)	4(100)	0(00)	0(00)	2(50)	2(100)	10(28.6)
	Negative	1	8(40)	2(100)	0(00)	1(100)	2(100)	1(25)	0(00)	14(40.0)
Esterase	Strong	≤0.63	7(35)	0(00)	1(25)	0(00)	0(00)	1(25)	1(50)	10(28.6)
	Weak	0.64–0.99	9(45)	0(00)	2(50)	0(00)	0(00)	2(50)	0(00)	13(37.1)
	Negative	1	4(20)	2(100)	1(25)	1(100)	2(100)	1(25)	1(50)	12(34.3)
Coagulase	Positive	N/A*	13(65)	1(50)	2(50)	0(00)	0(00)	2(50)	0(00)	18(51.4)
	Negative	N/A*	7(35)	1(50)	2(50)	1(100)	2(100)	2(50)	2(100)	17(48.6)
Biofilm	Strong	> 0.524	9(45)	0(00)	2(50)	1(100)	1(50)	0(00)	0(00)	13(37.1)
	Moderate	0.263–0.524	3(15)	0(00)	1(25)	0(00)	0(00)	2(50)	2(100)	8(22.9)
	Weak	0.132–0.262	1(05)	0(00)	1(25)	0(00)	1(50)	1(25)	0(00)	4(11.4)
	No biofilm formation	≤0.131	7(35)	2(100)	0(00)	0(00)	0(00)	1(25)	0(00)	10(28.6)

N/A* = Activity index cut off value not applicable for Coagulase test since the test was qualitative.

Table 3
Optical density measurements of biofilm formation by *Candida* species in microtiter plates.

Isolate ID	*Candida* species	Optical density values obtained from duplicate isolates on three separate occasions						mean optical density value	biofilm formation score
		occasion 1		occasion 2		occasion 3			
		duplicate 1	duplicate 2	duplicate 1	duplicate 2	duplicate 1	duplicate 2		
BF 1	*C. albicans*	0.572	0.591	0.52	0.58	0.491	0.59	0.557	strong
BF 2	*C. albicans*	0.14	0.13	0.12	0.14	0.13	0.11	0.128	negative
BF 3	*C. albicans*	0.21	0.24	0.18	0.22	0.185	0.177	0.202	weak
BF 4	*C. albicans*	0.54	0.53	0.56	0.56	0.62	0.58	0.565	strong
BF 5	*C. albicans*	0.58	0.54	0.56	0.53	0.542	0.543	0.549	strong
BF 6	*C. albicans*	0.52	0.48	0.44	0.51	0.49	0.424	0.477	moderate
BF 7	*C. albicans*	0.51	0.52	0.438	0.52	0.439	0.42	0.475	moderate
BF 8	*C. albicans*	0.48	0.49	0.51	0.58	0.582	0.563	0.534	strong
BF 9	*C. albicans*	0.11	0.12	0.12	0.121	0.12	0.1	0.115	negative
BF 10	*C. albicans*	0.25	0.24	0.26	0.347	0.342	0.345	0.297	moderate
BF 11	*C. albicans*	0.12	0.112	0.099	0.096	0.108	0.13	0.111	negative
BF 12	*C. albicans*	0.5	0.54	0.55	0.549	0.549	0.54	0.538	strong
BF 13	*C. albicans*	0.54	0.51	0.548	0.55	0.543	0.543	0.539	strong
BF 14	*C. albicans*	0.13	0.13	0.13	0.14	0.12	0.11	0.127	negative
BF 15	*C. albicans*	0.123	0.128	0.18	0.121	0.12	0.11	0.130	negative
BF 16	*C. albicans*	0.098	0.099	0.14	0.139	0.128	0.093	0.116	negative
BF 17	*C. albicans*	0.541	0.542	0.551	0.552	0.552	0.537	0.546	strong
BF 18	*C. albicans*	0.12	0.14	0.11	0.105	0.135	0.142	0.125	negative
BF 19	*C. albicans*	0.582	0.552	0.577	0.54	0.567	0.62	0.573	strong
BF 20	*C. albicans*	0.643	0.645	0.563	0.56	0.644	0.617	0.612	strong
BF 21	*C. dubliniensis*	0.122	0.125	0.114	0.12	0.13	0.13	0.124	negative
BF 22	*C. dubliniensis*	0.106	0.109	0.128	0.13	0.12	0.13	0.119	negative
BF 23	*C. glabrata*	0.648	0.645	0.663	0.66	0.644	0.672	0.655	strong
BF 24	*C. glabrata*	0.134	0.132	0.136	0.152	0.156	0.148	0.143	weak
BF 25	*C. glabrata*	0.443	0.505	0.503	0.52	0.48	0.47	0.487	moderate
BF 26	*C. glabrata*	0.64	0.62	0.663	0.56	0.644	0.627	0.6257	strong
BF 27	*C. lusitaniae*	0.841	0.645	0.763	0.756	0.844	0.718	0.7612	strong
BF 28	*C. parapsilosis*	0.242	0.245	0.243	0.216	0.244	0.218	0.235	weak
BF 29	*C. parapsilosis*	0.583	0.585	0.563	0.556	0.544	0.508	0.557	strong
BF 30	*C. tropicalis*	0.124	0.122	0.114	0.118	0.123	0.124	0.121	negative
BF 31	*C. tropicalis*	0.52	0.5	0.438	0.52	0.439	0.48	0.483	moderate
BF 32	*C. tropicalis*	0.164	0.182	0.19	0.192	0.164	0.611	0.251	weak
BF 33	*C. tropicalis*	0.284	0.285	0.282	0.284	0.276	0.278	0.282	moderate
BF 34	*C. krusei*	0.443	0.445	0.462	0.461	0.444	0.442	0.450	moderate
BF 35	*C. krusei*	0.348	0.352	0.356	0.356	0.344	0.409	0.361	moderate

All isolates were subjected to duplicate testing on three distinct occasions to assess biofilm formation (BF) using microtiter plates (mtp). The optical density (OD) of the mtp was measured spectrophotometrically using a BIOTEK Synergy multimode reader. The OD values obtained from the duplicate isolates (designated as duplicate 1 and duplicate 2) across the three testing occasions (labelled as occasion 1, occasion 2, and occasion 3) were averaged. These mean OD values were subsequently utilized for categorizing biofilm formation, with the following interpretations applied: no biofilm formation for mean OD values $\leq$ 131, weak biofilm formation for mean OD values ranging from 132 to 0.262, moderate biofilm formation for mean OD values between 0.263 and 0.524, and strong biofilm formation for mean OD values exceeding 0.524.

Biofilm formation was noted in 25/35 (71.4 %) of the total isolates and in 13/20 (65 %) and 12/15 (80 %) of the *C. albicans* and NAC isolates, respectively. Additionally, 4/4 (100 %) *C. glabrata*, 1/1 (100 %) *C. lusitaniae,* 1/2 (50 %) *C. dubliniensis*, 1/2 (50 %) *C. parapsilosis*, 2/4 (50 %) *C tropicalis*, and 1/2 (50 %) *C. krusei* isolates were biofilm producers. Furthermore, 1/1 (100 %) *C. lusitaniae*, 2/4 (50) *C. glabrata,* 1/2 (50 %) *C. parapsilosis,* 9/20 (45 %) *C. albicans*, and 7/20 (35 %) *C. albicans* strains were strong biofilm producers (Table 2), (Table 3)

4. Discussion

This study provides critical insights into the virulence characteristics of *Candida* species isolated from people living with HIV (PLHIV) with OPC infection in Uganda. With the high prevalence of OPC among PLHIV, understanding the pathogenic potential of various *Candida* species is essential, especially given the observed shift from *Candida* albicans to NAC species in recent years. Our analysis of phospholipase, proteinase, hemolysin, esterase, coagulase activities, and biofilm formation in *Candida* isolates reveals critical aspects of their virulence and adaptability in immunocompromised hosts.

The detection of phospholipase activity in 94.3 % of the isolates, with a notable 100 % in *C. albicans*, highlights the enzyme's significance as a major virulence factor during OPC pathogenesis. Phospholipases have the capacity to disrupt host cell membranes, thereby enhancing tissue invasion and facilitating nutrient acquisition. This phenomenon is particularly pertinent in individuals with compromised immune systems [39]. The pronounced activity of phospholipase among *C. albicans* isolates aligns with results from prior research, which indicates a strong correlation between phospholipase enzymatic activity and the pathogenic potential of *C. albicans* [40,41]. This correlation may be attributable to the germ tube induction capacity of *C. albicans*, which promotes mucosal penetration and enables the organism to secure nutrients from the host at an early stage of the OPC infection process [21].

Proteinase activity was also prevalent, detected in 88.6 % of the isolates. This enzyme facilitates the breakdown of host proteins, thereby aiding in tissue invasion and immune evasion [39]. The observed higher activity in *C. albicans* compared to NAC species *C. glabrata*, *C tropicalis,* and *C. krusei* isolates may reflect the adaptive strategies employed by *C. albicans* to thrive in hostile environments, such as the oral cavity of immunocompromised individuals. The impact of factors such as low pH and xerostomia on proteinase production in PLHIV may further accentuate the greater proteinase activity in *C. albicans* [42]. This finding agrees with previous studies by Deepa et al. [43], who reported greater proteinase activity in *C. albicans* (90 %) than in NAC isolates (81.3 %), and Mane et al. [31], who reported proteinase activity in 87.8 % of *C. albicans* isolates.

Our results indicate a notable hemolytic activity of up to 60 % of all isolates. This prevalence is consistent across both *C. albicans* and NAC

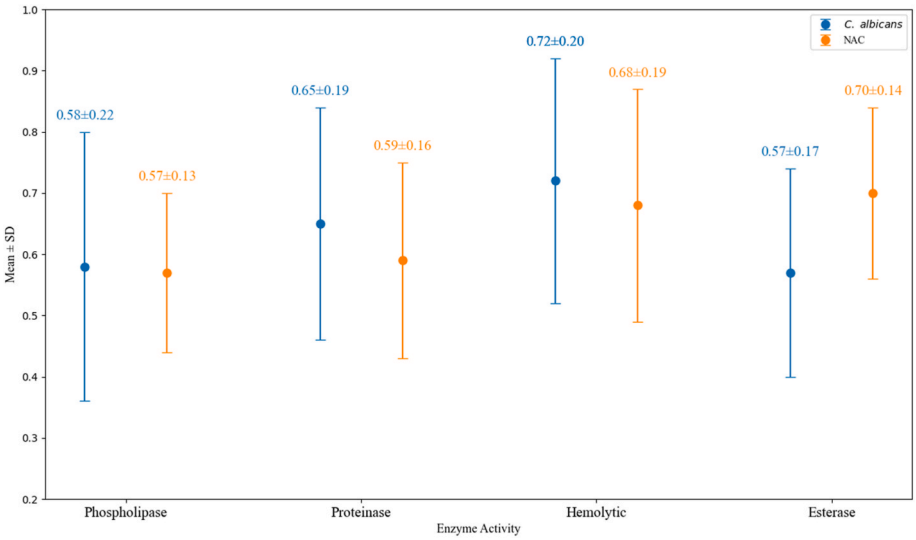


Fig. 3. ***Comparison of different extracellular hydrolytic enzymatic activity mean values with respective standard deviations of C. albicans NAC isolates***
The independent student's *t*-test analysis of enzymatic activities in *C. albicans* and NAC isolates showed no significant differences in phospholipase, proteinase, and hemolytic activities between *C. albicans* and NAC isolates. The mean phospholipase activity for *C. albicans* was 0.58 ± 0.22 and for NAC was 0.57 ± 0.13 (*p*-value 0.88). The mean proteinase activity was 0.65 ± 0.19 for *C. albicans* and 0.59 ± 0.16 for NAC (*p*-value 0.37). Hemolytic activity results showed a mean of 0.72 ± 0.20 for *C. albicans* and 0.68 ± 0.19 for NAC (*p*-value 0.65). However, a significant difference was noted in esterase activity, with *C. albicans* showing a mean of 0.57 ± 0.17 compared to NAC's 0.70 ± 0.14 (*p*-value 0.04).

species, indicating a mechanism for iron acquisition, which is vital for and a common trait among *Candida* species. This may be because *C. albicans* and NAC have the same mechanism of hemolytic activity by secreting the hemolysin enzyme that lyses host erythrocytes and obtains iron for their own metabolism and survival, facilitating host tissue invasion. Additionally, candidalysin toxin contributes to red blood cell lysis in *C. albicans* infections [31,44]. The observed hemolytic activity in 100 % of *C. glabrata* and C. *krusei* isolates, as well as 75 % of *C. tropicalis* isolates, highlights the potential pathogenicity of these species, which are increasingly recognized as significant contributors to OPC among PLHIV. Our findings agree with those of a study by Fatahinia et al. [35], who reported that 100 % of *C. glabrata* and *C. krusei* were hemolysin producers, whereas Tsang et al. [45] reported that 100 % of *C. albicans* strains had hemolytic activity.

Esterase activity was detected in 65.7 % of the total isolates, with a notably higher prevalence in *C. albicans* (80 %) compared to NAC isolates (46.7 %). This disparity aligns with existing literature that suggests *C. albicans* possesses a more robust enzymatic virulence attributes, which may confer a competitive advantage in terms of pathogenicity. The observation that 75 % of *C. glabrata* and *C. tropicalis* isolates also exhibited esterase activity suggesting that these NAC species may not be as benign as previously thought and could contribute to significant OPC clinical challenges, particularly in immunocompromised patients. Esterase enzymes have the ability to hydrolyze ester bonds in the host cell, enhancing binding to the host cell, penetration, and invasion [21]. Interestingly, the absence of esterase activity in *C. dubliniensis*, *C. lusitaniae*, and *C. parapsilosis* raises questions about the evolutionary adaptations and pathogenic mechanisms of these species. It is possible that these species have developed alternative strategies for host invasion that do not rely on esterase activity. The lack of esterase production could also indicate a lower level of virulence, which is consistent with clinical observations that these species are less frequently associated with OPC infections depending on the stage of infection and the host immune response compared to *C. albicans* and certain NAC species [46,47]. Our results are in agreement with those of previous studies demonstrating stronger esterase activity in *C. albicans* than in NAC [46,47]. In contrast to our results, Pandey et al. [21] reported no esterase activity in *C. glabrata* or *C. krusei,* whereas Fatahinia et al. [35] reported esterase activity in 100 % of *C. albicans, C. glabrata*, *C. krusei* and *C. dubliniensis*.

Coagulase activity was particularly pronounced in *C. albicans*, where 65 % of isolates demonstrated coagulase activity, while non-*albicans Candida* (NAC) species exhibited a lower coagulase activity at 33.3 %. These findings underscore the heterogeneity in coagulase activity among different *Candida* species and suggest potential implications for their pathogenicity and interactions with host immune responses. The variability in coagulase activity among the different *Candida* species in our study may reflect intrinsic differences in their pathogenic mechanisms and virulence factors. For instance, the 50 % coagulase activity observed in *C. dubiniensis*, *C. glabrata*, and *C. tropicalis* isolates suggest that these species may possess similar virulence traits that enable them to establish infections more effectively than those species that exhibited no coagulase activity, such as *C. lusitaniae*, *C. parapsilosis*, and *C. krusei*. This finding aligns with the notion that *C. albicans*, known for its higher pathogenic potential, exhibits enhanced virulence factors, including coagulase production, which may contribute to its prevalence as a leading cause of OPC among PLHIV. Coagulase binds to fibrinogen and activates prothrombin to convert fibrinogen to fibrin, leading to the clotting of plasma, which protects *Candida* species from being phagocytosed by granulocytes [22]. This result is consistent with a study by Yigit et al. [37], who reported similar coagulant activity in *C. albicans* (64.7 %). However, our findings disagree with those of Gupta et al. [48], who reported low coagulase activity in both *C. albicans* (25.9 %) and *C. tropicalis* (28.8 %). The variability in coagulase activity could be due to differences in host immune statuses as well as differences in coagulase activity detection techniques, as we used rabbit plasma since it has been indicated that rabbit plasma has greater sensitivity to coagulase activity in *Candida* species than sheep and human plasma does [37].

The observed biofilm formation in 71.4 % of the total isolates, with a notable increased formation in NAC) species (80 %) compared to *C. albicans* (65 %), highlights the potential clinical implications of these *Candida* pathogens in OPC among PLHIV. The NAC species may possess enhanced capabilities for biofilm formation, which could contribute to their increasing prevalence as pathogens in immunocompromised hosts. Biofilms are complex communities of microorganisms that adhere to surfaces and are encased in a protective extracellular matrix. This biofilm architecture provides a significant advantage for *Candida* species, allowing them to withstand antifungal treatments and evade host

immune responses [23]. The protection conferred by biofilms is particularly relevant in the oral cavity, where the presence of saliva, mucosal surfaces, and fluctuating pH levels can create a challenging environment for microbial survival. The ability of *Candida* species to form biofilms in this niche not only enhances their survival but also facilitates recurrence of OPC infection, leading to treatment failures and persistent symptoms of OPC. The results indicate that all *C. glabrata, C. lusitaniae, C. parapsilosis*, and *C. krusei* isolates demonstrated biofilm production. This is particularly concerning given that *C. glabrata* has been increasingly recognized as a significant pathogen in OPC, especially among immunocompromised individuals. The high biofilm production rates observed in these NAC species may be attributed to their distinct cell surface properties and adhesion mechanisms. For instance, the ability of these species to adhere to epithelial cells and form robust biofilms may be influenced by differences in cell wall composition, morphology, and gene expression profiles, as suggested by previous studies [49,50].

4.1. Limitations

This study acknowledges limitations due to a small sample size derived from our previous larger study [26] which included 35 isolates from 29 individuals with HIV and oropharyngeal candidiasis (OPC). This reduced sample may have led to inadequate statistical power for comparing virulence attributes among NAC species, particularly due to limited NAC species representation and the lack of control groups with HIV-negative individuals without OPC.

While methods like the McFarland standard and crystal violet staining offer quick estimates of *Candida* cell density and biofilm formation, respectively, their insufficient contrast in studies of *Candida* virulence limitations may have affected the findings' accuracy. The study advocates for future research to adopt additional techniques to improve the investigation of *Candida* virulence. Furthermore, the study highlights the importance of longitudinal data to understand enzymatic activity dynamics related to antiviral therapy or disease progression, but its cross-sectional design and resource constraints prevented follow-up assessments.

5. Conclusion

Phospholipases and proteinases had the strongest activities in oropharyngeal *Candida* species. A significant difference was noted in the mean esterase values of the *C. albicans* (and NAC isolates.

CRediT authorship contribution statement

Benson Musinguzi: Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Andrew Akampurira:** Resources, Funding acquisition, Data curation. **Hope Derick:** Formal analysis, Data curation. **Laban Turyamuhika:** Methodology, Formal analysis, Data curation. **Alex Mwesigwa:** Methodology, Formal analysis, Data curation. **Edson Mwebesa:** Writing – review & editing, Formal analysis. **Vicent Mwesigye:** Writing – review & editing, Methodology, Data curation. **Immaculate Kabajulizi:** Writing – review & editing, Formal analysis, Data curation. **Tahalu Sekulima:** Resources, Formal analysis, Data curation. **Francis Ocheng:** Writing – review & editing, Supervision, Conceptualization. **Herbert Itabangi:** Writing – review & editing, Supervision, Conceptualization. **Gerald Mboowa:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Obondo James Sande:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Beatrice Achan:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Ethical approval and consent

This study was approved by the Makerere University School of Biomedical Sciences Research Ethics Committee (Reference; SBS-2022-254) and Uganda National Council for Science and Technology (Reference; UNCST-HS3823ES). Written informed consent was obtained from the study participants before being enrolled in the study.

Availability of data and materials

The analyzed datasets are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

[1] O. Uwishema, et al., The syndemic burden of HIV/AIDS in Africa amidst the COVID-19 pandemic, Immun Inflamm Dis 10 (1) (2022) 26–32, https://doi.org/10.1002/iid3.544.
[2] S. Patil, B. Majumdar, S.C. Sarode, G.S. Sarode, K.H. Awan, Oropharyngeal candidosis in HIV-infected patients-an update, Front. Microbiol. 9 (MAY) (2018) 1–9, https://doi.org/10.3389/fmicb.2018.00980.
[3] L.O. Sánchez-Vargas, et al., Oral *Candida* isolates colonizing or infecting human immunodeficiency virus-infected and healthy persons in Mexico, J. Clin. Microbiol. 43 (8) (2005) 4159–4162, https://doi.org/10.1128/JCM.43.8.4159-4162.2005.
[4] L. De Repentigny, D. Lewandowski, P. Jolicoeur, Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection, Clin. Microbiol. Rev. 17 (4) (2004) 729–759, https://doi.org/10.1128/CMR.17.4.729-759.2004.
[5] P. Anwar Khan, A. Malik, H. Subhan Khan, Profile of candidiasis in HIV infected patients, Iran. J. Microbiol. 4 (4) (2012) 204–209.
[6] P. Miguel, et al., Multi-drug resistant (MDR) oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon, Diagn. Microbiol. Infect. Dis. 79 (2) (2013) 222–227, https://doi.org/10.1016/j.diagmicrobio.2013.09.016.
[7] N.F. Ambe, et al., The prevalence, risk factors and antifungal sensitivity pattern of oral candidiasis in hiv/aids patients in Kumba District Hospital, South West Region, Cameroon, Pan African Medical Journal 36 (May 2020) 1–14, https://doi.org/10.11604/pamj.2020.36.23.18202.
[8] K. Yongabi, W. Mbacham, K.A. Yongabi, W.F. Mbacham, K.K. Nubia, R.M. Singh, Yeast strains isolated from HIV-seropositive patients in Cameroon and their sensitivity to extracts of eight medicinal plants, Article in African Journal of

Microbiology Research 3 (4) (2009) 133–136 [Online]. Available: https://www.researchgate.net/publication/237424464.
[9] P. Miguel, et al., Multi-drug resistant (MDR) oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon, Diagn. Microbiol. Infect. Dis. 79 (2) (2013) 222–227, https://doi.org/10.1016/j.diagmicrobio.2013.09.016.
[10] C. Enwuru, et al., Fluconazole Resistant Opportunistic Oro-Pharyngeal *candida* and Non-*candida* Yeast-like Isolates from HIV Infected Patients Attending ARV Clinics in Lagos, Nigeria, 2008.
[11] E.I. Nweze, U.L. Ogbonnaya, Oral *Candida* isolates among HIV-infected subjects in Nigeria, J. Microbiol. Immunol. Infect. 44 (3) (Jun. 2011) 172–177, https://doi.org/10.1016/j.jmii.2011.01.028.
[12] Rita O. Oladele, Iriagbonse I. Osaigbovo, Patrick V. Lofor, Fluconazole resistance among oral *Candida* isolates from people living with HIV/AIDS in a Nigerian tertiary hospital, j.fungi 3 (2017) 69, https://doi.org/10.3390/jof3040069, 10.3390ljof3040069, www.mdpi.com/journal/jof, 2017.
[13] C.C. Ekwealor, C.J. Nweke, C.G. Anaukwu, V.N. Anakwenze, C.M. Ogbukagu, A. N. Mba, Prevalence and antifungal susceptibility pattern of oral candidiasis among HIV-infected patients in a Mission Hospital, southeast Nigeria, Afr. J. Clin. Exp. Microbiol. 24 (3) (Jul. 2023) 289–298, https://doi.org/10.4314/ajcem.v24i3.9.
[14] F. Kwamin, N.O. Nartey, F.S. Codjoe, M.J. Newman, Distribution of *Candida* species among HIV-positive patients with oropharyngeal candidiasis in Accra, Ghana, J Infect Dev Ctries 7 (1) (2013) 41–45, https://doi.org/10.3855/jidc.2442.
[15] A. Konaté, et al., Oropharyngeal candidiasis prevalence among HIV-infected patients at the teaching hospital of Treichville (Abidjan, Côte d'Ivoire), J. Mycol. Med. 27 (4) (2017) 549–553, https://doi.org/10.1016/j.mycmed.2017.08.005.
[16] M. Benson, et al., Distribution and antifungal susceptibility profile of oropharyngeal *Candida* species isolated from people living with HIV in the era of universal test and treat policy in Uganda, Ther Adv Infect Dis 11 (Jan) (2024), https://doi.org/10.1177/20499361241255261.
[17] E. Agwu, J.C. Ihongbe, B.A. McManus, G.P. Moran, D.C. Coleman, D.J. Sullivan, Distribution of yeast species associated with oral lesions in HIV-infected patients in Southwest Uganda, Med. Mycol. 50 (3) (Apr. 2012) 276–280, https://doi.org/10.3109/13693786.2011.604862.
[18] P. Ed, T. Young, H. Holmes, P. Ed, T. Young, H. Holmes, Interventions for the prevention and management of oropharyngeal candidiasis associated with HIV infection in adults and children (Review), www.cochranelibrary.com, 2010.
[19] L. Taverne-Ghadwal, et al., Epidemiology and prevalence of oral candidiasis in HIV patients from Chad in the post-HAART era, Front. Microbiol. 13 (February) (2022), https://doi.org/10.3389/fmicb.2022.844069.
[20] N. Pandey, M.K. Gupta, R. Tilak, Extracellular hydrolytic enzyme activities of the different *Candida* spp. isolated from the blood of the Intensive Care Unit-admitted patients, J Lab Physicians 10 (4) (Oct. 2018) 392–396, https://doi.org/10.4103/jlp.jlp_81_18.
[21] M. Schaller, C. Borelli, H.C. Korting, B. Hube, Hydrolytic enzymes as virulence factors of *Candida* albicans, Mycoses 48 (6) (2005) 365–377, https://doi.org/10.1111/j.1439-0507.2005.01165.x.
[22] C. Fehrmann, et al., Role for the fibrinogen-binding proteins Coagulase and Efb in the Staphylococcus aureus-*Candida* interaction, International Journal of Medical Microbiology 303 (5) (2013) 230–238, https://doi.org/10.1016/j.ijmm.2013.02.011.
[23] M. Staniszewska, Virulence factors in *Candida* species, Curr. Protein Pept. Sci. 21 (3) (2019) 313–323, https://doi.org/10.2174/1389203720666190722152415.
[24] M. Mroczyńska, A. Brillowska-Dabrowska, Virulence of clinical *candida* isolates, Pathogens 10 (4) (Apr. 2021), https://doi.org/10.3390/pathogens10040466.
[25] E. Agwu, J.C. Ihongbe, B.A. McManus, G.P. Moran, D.C. Coleman, D.J. Sullivan, Distribution of yeast species associated with oral lesions in HIV-infected patients in Southwest Uganda, Med. Mycol. 50 (3) (2012) 276–280, https://doi.org/10.3109/13693786.2011.604862.
[26] M. Benson, et al., Distribution and antifungal susceptibility profile of oropharyngeal *Candida* species isolated from people living with HIV in the era of universal test and treat policy in Uganda, Ther Adv Infect Dis 11 (2024), https://doi.org/10.1177/20499361241255261.
[27] E. Agwu, J.C. Ihongbe, B.A. McManus, G.P. Moran, D.C. Coleman, D.J. Sullivan, Distribution of yeast species associated with oral lesions in HIV-infected patients in Southwest Uganda, Med. Mycol. 50 (3) (2012) 276–280, https://doi.org/10.3109/13693786.2011.604862.
[28] O. Bader, M. Weig, L. Taverne-Ghadwal, R. Lugert, U. Groß, M. Kuhns, Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry, Clin. Microbiol. Infection 17 (9) (2011) 1359–1365, https://doi.org/10.1111/j.1469-0691.2010.03398.x.
[29] L.P. Samaranayake, J.M. Raeside, T.W. Macfarlane, Factors affecting the phospholipase activity of *candida* species in vitro, Med. Mycol. 22 (3) (1984) 201–207, https://doi.org/10.1080/00362178485380331.
[30] M.F. Price, A.N.D. Wilkinson, L.O. Gentry, Plate M E T H O D for Detection of P H O S P H O L I P a S E Activity in *Candida* a L B I C a N S, 1982, pp. 7–14. April 1981.
[31] A. Mane, S. Gaikwad, S. Bembalkar, A. Risbud, "Increased Expression of Virulence Attributes in Oral *Candida Albicans* Isolates from Human Immunodeficiency Virus-Positive Individuals Printed in Great Britain,", 2012, pp. 285–290, https://doi.org/10.1099/jmm.0.036269-0.
[32] R. De Paula Menezes, É.B. De Melo Riceto, A.S. Borges, D.V.D. De Brito Röder, R. Dos Santos Pedroso, Evaluation of virulence factors of Candida albicans isolated from HIV-positive individuals using HAART, Arch. Oral Biol. 66 (Jun. 2016) 61–65, https://doi.org/10.1016/j.archoralbio.2016.02.004.
[33] F. Staib, Serum–Proteins as Nitrogen Fungi, Sabouraudia, 1965.
[34] J.M. Manns, D.M. Mosser, H.R. Buckley, Production of a hemolytic factor by *Candida albicans*, Infect. Immun. 62 (11) (1994) 5154–5156, https://doi.org/10.1128/iai.62.11.5154-5156.1994.
[35] M. Fatahinia, F. Poormohamadi, A.Z. Mahmoudabadi, Comparative study of esterase and hemolytic activities in clinically important *Candida* species, isolated from oral cavity of diabetic and non-diabetic individuals, Jundishapur J. Microbiol. 8 (3) (2015) 3–6, https://doi.org/10.5812/jjm.20893.
[36] M. Slifkin, Tween 80 opacity test responses of various *Candida* species 38 (12) (2000) 4626–4628.
[37] N. Yigit, E. Aktas, S. Dagistan, A. Ayyildiz, Investigating biofilm production, coagulase and hemolytic activity in *Candida* species isolated from denture stomatitis patients, Eurasian J Med 43 (1) (2011) 27–32, https://doi.org/10.5152/eajm.2011.06.
[38] S. Kırmusaoğlu, The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents, IntechOpen, 2019.
[39] F. Fathi, A.Z. Mahmoudabadi, M. Fatahinia, A comparative study on the production of extracellular hydrolytic enzymes of *C. albicans* and non-albicans *Candida* species isolated from HIV+/AIDS patients and healthy individuals, Curr Med Mycol 8 (2) (Jun. 2022) 32–39, https://doi.org/10.18502/cmm.8.2.10330.
[40] W.P. da Silva-Rocha, et al., *Candida* species distribution, genotyping and virulence factors of *Candida albicans* isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil, BMC Oral Health 14 (1) (2014) 1–9, https://doi.org/10.1186/1472-6831-14-20.
[41] C.P.G. Kumar, S.S.J. Kumar, T. Menon, Phospholipase and proteinase activities of clinical isolates of *Candida* from immunocompromised patients, Mycopathologia 161 (4) (2006) 213–218, https://doi.org/10.1007/s11046-005-0157-4.
[42] T. Wu, L. Samaranayaket, B.Y. Cao, J. Wang, In-vitro proteinase production by oral *Candida albicans* isolates from individuals with and without HIV infection and its attenuation by antimycotic agents 44 (1996).
[43] D. K, J. T, M. A, In vitro evaluation of virulence factors of *Candida* species isolated from oral cavity, J. Microbiol. Antimicrob. 7 (3) (2015) 28–32, https://doi.org/10.5897/jma2015.0337.
[44] R.D. Rossoni, Comparison of the hemolytic activity between *C . albicans* and non-albicans Candida species 27 (6) (2013) 484–489.
[45] C.S.P. Tsang, F.C.S. Chu, W.K. Leung, L.J. Jin, L.P. Samaranayake, S.C. Siu, Phospholipase, proteinase and haemolytic activities of *Candida albicans* isolated from oral cavities of patients with type 2 diabetes mellitus, J. Med. Microbiol. 56 (10) (2007) 1393–1398, https://doi.org/10.1099/jmm.0.47303-0.
[46] H. Nouraei, M.G. Jahromi, L.R. Jahromi, K. Zomorodian, K. Pakshir, Potential pathogenicity of *Candida* species isolated from oral cavity of patients with diabetes mellitus 2021 (type 1) (2021) 1–6.
[47] S.H. Subramanya, et al., Antifungal susceptibility and phenotypic virulence markers of *Candida* species isolated from Nepal, BMC Res. Notes 10 (1) (2017) 1–7, https://doi.org/10.1186/s13104-017-2852-x.
[48] D.J. Gupta, H. Shah, 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 3 (2) (2017) 188–194, https://doi.org/10.17511/jopm.2017.i02.20.
[49] M. Negri, M. Henriques, D.W. Williams, J. Azeredo, Adherence and biofilm formation of non- *Candida albicans Candida* species 19 (5) (2011) 241–247, https://doi.org/10.1016/j.tim.2011.02.003.
[50] F.M. Sariguzel, E. Berk, A.N. Koc, H. Sav, G. Demir, "Investigation of the Relationship between Virulence Factors and Genotype of *Candida* Spp . Isolated from Blood Cultures Original Article Investigation of the Relationship between Virulence Factors and Genotype of *Candida* Spp . Isolated from Blood Cultures,", August, 2015, https://doi.org/10.3855/jidc.5359.