FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 4, April, 2025, pp 66 - 73
DOI: https://doi.org/10.33003/fjs-2025-0904-3548

CANDIDA SPECIES FROM HUMAN MUCOSAL SURFACES: PREVALENCE, VIRULENCE FACTORS AND SUSCEPTIBILITY TO ITRACONAZOLE

***[1]Olajide J. Akinjogunla, [2]Adebowale T. Odeyemi, [3]Ekom N. Edem, [4]Ngozichukwu G. Uzoewulu, [5]Faith C. Ogboona, [1]Christiana R. Otu and [1]Florence O. Uko**

[1]Department of Microbiology, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.
[2]Department of Microbiology, Landmark University SDG Groups 2 and 12, Omu-Aran, Kwara State, Nigeria.
[3]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, University of Uyo, Akwa Ibom State, Nigeria.
[4]Department of Medical Microbiology and Parasitology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.
[5]Department of Microbiology, School of Biological Sciences, Federal University of Technology, Owerri, Imo State, Nigeria

*Corresponding authors' email: papajyde2000@yahoo.com
ORCID iD: https://doi.org/0000-0001-7105-6771

ABSTRACT

Candida species are found throughout the human population as both commensal organisms and intermittent pathogens. The prevalence, virulence factors, and Itraconazole susceptibility of *Candida* spp. from the mucosal surfaces were determined using the standard technique, mycological media, and disc diffusion method, respectively. *Candida albicans*, *Candida dubliniensis*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, and *Candida parapsilosis* were obtained. Nineteen (19) *Candida* spp. were found in the oral cavities, while the percentages of occurrence of *Candida* spp. in the nasal cavities were *C. albicans* (17.9%), *C. glabrata*, *C. krusei* (10.7%), *C. tropicalis* (7.1%), and *C. dubliniensis* (3.6%). Six Candida isolates were lipase producers; ≤ 80% of *C. albicans* and *C. krusei* produced haemolysin; and 20.0% to 28.6% were positive for phospholipase and amylase production. The percentage occurrence of caseinase-, gelatinase-, and biofilm-producing *Candida* spp. was 25.7%, 31.4%, and 20.0%, respectively. The coagulase-producing strains were *C. albic*ans (n = 2), *C. glabrata* (n = 1), and *C. tropicalis* (n = 1). Itraconazole (5 mg/mL) inhibited > 90% of *Candida* spp., while all the *C. tropicalis* and *C. parapsilosis* displayed resistance to 2.5 mg/mL of Itraconazole. The MIC values of Itraconazole ranged from 1.25 µg/mL to > 10 µg/mL, and the highest and lowest MFC obtained was 20 µg/mL and 2.5 µg/mL, respectively. The regression values of the ITR and ZIDs as exhibited by the isolates ranged from 0.6961 to 1.0. This study has provided updated data on the presence of pathogenic *Candida* spp. on mucosal surfaces and also revealed their susceptibility to Itraconazole.

Keywords: Occurrence, Candida, Virulence Factors, Itraconazole, Resistance

INTRODUCTION

Candida species are fungi that are widely found throughout the human population as both commensal organisms and intermittent pathogens and exist harmlessly on mucosal surfaces including those of the mouth, nose, vagina, and other anatomic locations (Calderone, 2002; Rafat *et al*., 2017). *Candida* spp. are found in the oral microbial flora of 2–70% of seemingly healthy individuals (Samaranayake and MacFarlane, 1990). Candidal colonization and infections occur when the usual equilibrium of the human body is disturbed (Calderone, 2002).

Expression of adhesins, invasins, thigmotropism, antigenic modulations, phenotypic switching, biofilm formation (Sardi *et al*., 2013; Akinjogunla *et al*., 2019), and the production of extracellular hydrolytic enzymes (EHEs) are significant virulence factors that contribute to the pathogenesis of candidal infection. The EHEs such as lipase, proteases, phospholipases, and haemolysin act synergistically under favourable conditions (Silva *et al*., 2011) and play an essential role in candidal overgrowth and facilitate adherence (Akinjogunla *et al*., 2018), tissue penetration, and invasion of the host tissue (Schaller *et al*., 2005; Tsang *et al*., 2007). The expression of the virulence factors among the Candida species may vary depending on the species, site and stage of infection, and host reaction (Sardi *et al*., 2013).

Variabilities in the patterns of sensitivity of *Candida* spp. to antifungal agents have been reported (Pfaller and Diekema, 2012). In many developing countries, especially Nigeria, routine testing for antifungal susceptibility of *Candida* species is not usually undertaken, and consequently, the data on the sensitivity of *Candida* spp. to Amphotericin B, Itraconazole, Nystatin, and Fluconazole is still extremely sparse. Similarly, emergent resistant populations of *Candida* spp. treated with antifungals have been reported (Da-Costa *et al*., 2009; Sun *et al*., 2009). Thus, it is vital to determine the patterns of antifungal susceptibility of *Candida* isolates in this era of moderate availability of antifungal agents. The main concept of this study was to investigate *Candida* spp. in the mucosal surfaces (nose and mouth) and determine their virulence factors and susceptibility to Itraconazole.

MATERIALS AND METHODS

Collection of Samples: A total of one hundred (100) samples, comprising oral (n = 50) and nasal (n = 50) samples from apparently healthy undergraduate students, were aseptically collected using sterile swab sticks from November to December, 2021. Prior to collecting samples, each student provided verbal informed consent. The oral and nasal samples were collected from students who had not received antifungal drugs for the previous five days, and were appropriately labelled, kept on ice immediately, and transported to the microbiology laboratory for mycological analyses.

Mycological Analysis of Oral and Nasal Swabbed Samples: The oral and nasal swabbed samples were inoculated into the

peptone water for 4-6 h and were later streaked onto plates of Sabouraud Dextrose Agar (SDA, Oxoid) supplemented with chloramphenicol (CHL, 10 µg) and incubated at 35ºC± 2 for 48 h. Thereafter, the plates were examined for candida growth, subcultured onto fresh plates of SDA supplemented with CHL, and incubated at 35ºC± 2 for 48 h. The pure Candida isolates were subcultured onto plates of CHROMagar Candida (Difco BBL., USA), incubated at 35°C ± 2 for 48 h, and pigmentation were observed and used for species differentiation. The Candida species maintained on SDA slant at 4ºC were also identified using Gram staining, germ tube formation, carbohydrate fermentation tests (Barnett and Hunter, 1987; Akinjogunla and Eghafona, 2012).

Identification of Candida Species using VITEK 2 Automated System: The *Candida* species were further identified by the VITEK 2 compact system (Vitek card YST ID (Ref.) 21343, Biomeriux Inc., France) according to the manufacturer's instructions. Briefly, *Candida* suspensions were prepared by mixing the colonies with 3 mL of Vitek saline (0.45%) to obtain the 0.5 McFarland turbidity standard. Thereafter, the test cards were loaded with the suspensions, sealed, and incubated for 18 h at 35°C using the VITEK 2 instrument. The identification of *Candida* species was achieved by reading cards and comparing with the database using the software version 8.01.

Determination of Virulence Factors: The methods described by Deorukhkar *et al*. (2014) and Akinjogunla *et al*. (2020) were used for the detection of virulence factors in *Candida* spp. Each plate of tributyrin agar, egg yolk agar, skim milk agar, gelatin agar, human blood-Sabouraud Dextrose agar, and starch agar was spot inoculated with 10 µL of Candida isolate suspension, adjusted to turbidity of 0.5 McFarland standards. All the plates were incubated at 35ºC ± 2 for 72 h. Amylase-producing Candida isolates were detected using starch agar. The Candida isolates were streaked onto starch agar plates and aerobically incubated for 48-72 h at 35°C ± 2. After incubation, 3 drops of 10 % Lugol's iodine were put on the culture plates and allowed to react for 10 min. Clear zones around the colonies indicated production of lipase, phosphokinase, caseinase, gelatinase, and haemolysin, respectively. The formation of coagulase and biofilm was determined using EDTA-human plasma (EDTA-HP) and Glucose-Sabouraud Dextrose Broth (GSDB), respectively. A loopful of Candida isolate was inoculated onto a tube of EDTA-HP and GSDB and incubated at 35°C ± 2 for 48 h. Each tube of EDTA-HP was observed for clot formation within 24 h, and the presence of a clot indicated coagulase production. The contents of each tube of GSDB were poured out, and each tube was washed with phosphate buffer saline, dried, and stained with safranin. A visible film lined the wall and bottom of the tube, indicating biofilm formation.

Source of Antifungal Drugs (Itraconazole): Itraconazole (ITR 100 mg, Genix Pharma Pvt. Ltd., Pakistan) was purchased in tablet form from standard pharmacy stores in Uyo. Stock solutions (10 $mgmL^{-1}$) of ITR were prepared using sterile distilled water (dH_2O) as the solvent and stored at 4°C prior to each experiment.

Anticandidal Activities of Itraconazole: *In vitro* anticandidal activities of Itraconazole (ITR) were evaluated by the disc diffusion method (CLSI, 2015; Okon *et al*., 2020; Akinjogunla *et al*., 2021). Nineteen Candida isolates, comprising *C. albicans (n = 6), C. dubliniensis (n = 2), C. tropicalis (n = 3), C. krusei (n = 3), C. glabrata (n = 4), and C. parapsilosis (n = 2), were used.* One millilitre of each *Candida* inoculum, prepared directly from a 48-h SDA plate and adjusted to the 0.5 McFarland Turbidity Standard, was inoculated onto each plate containing Mueller Hilton Agar (MHA) supplemented with 2% glucose and 0.5 g/mL methylene blue, and thereafter evenly spread using a sterile spreader. The ITR were dissolved in dH_2O to achieve graded concentrations of 2.5, 5.0, and 10 $mgmL^{-1}$. Each sterile filter paper disc (6 mm in diameter) was impregnated with 0.1 mL of 2.5, 5.0, and 10 $mgmL^{-1}$ of ITR. The impregnated discs were carefully placed on MHA plates that had previously been inoculated with the candida isolates and were incubated at 35°C ± 2 for 48 h. A disc containing 10 µL of 5% dimethyl sulfoxide (DMSO) that served as a control was included in each plate. The experiments were performed in triplicates, and the mean zones of inhibition diameter in millimeters were measured, recorded, and considered as an indication for anticandidal activity.

Determination of Minimum Inhibitory Concentration (MIC): The MIC values of ITR against *Candida* spp. were determined using broth microdilution (CLSI, 2015). One hundred milliliter (mL) of the stock solution (20 $mgmL^{-1}$ of ITR) was serially diluted in sterile dH_2O by two-fold dilution to achieve concentration ranges between 10 and 0.625 $mgmL^{-1}$. In each test tube, 100 µL of varying concentrations of ITR (0.625, 1.25, 2.5, 5, 10, and 20 $mgmL^{-1}$) and 9.9 mL of Sabouraud Dextrose broth were added to give the final concentrations in the range of 6.25–200 μgmL^{-1} for the MIC testing, and then, a loopful of each prepared candidal suspension was added to each test tube. A tube comprising dH_2O and inoculum candida cells served as a control. All the culture tubes were incubated at 35°C ± 2 for 48 h, and thereafter, the tubes were visibly examined for turbidity. The MIC was taken as the lowest concentration of the ITR that noticeably prevented candidal growth after 48 h of incubation.

Determination of Minimum Fungicidal Concentration (MFC): A loopful from each MIC broth tube, without visible candidal growth, was streak-inoculated onto freshly prepared SDA plates. The inoculated plates were incubated at 35°C ± 2 for 48 h, then examined for candidal growth. The MFC value was taken as the lowest concentration that killed 99.9% of the test *Candid*a spp. after 48 h of incubation at 37°C.

Statistical Analysis

The significance difference between the mean values was determined by the Duncan multiple range test (SPSS software, Window version 22), and a p-value of < 0.05 was considered statistically significant. The relationship between the different concentrations of ITR and the overall anticandidal activity, assessed as the diameters of inhibition zones with regard to the *Candida* species, was determined by linear regression analysis.

RESULTS AND DISCUSSION

The six species of Candida identified using morphological, germ tube, growth at 35°C and 45°C, carbohydrate fermentation and enzymatic tests were *Candida albicans, Candida dubliniensis, Candida tropicalis, Candida krusei, Candida glabrata, and Candida parapsilosis (Table 1). The C. albicans appeared blue-green, C. dubliniensis* was dark-green, *C. tropicalis* was dark-blue, *C. krusei* was purple, *C. glabrata* was white, *and C. parapsilosis developed pink colonies* on CHROM Agar™, a chromogenic medium (Table 1).

Nineteen (19) isolates of *Candida* spp. were recovered from the oral cavities (n = 28) of the undergraduate students. The 19 (67.9%) Candida isolates were *C. albicans* (n = 7, 25.0%), *C. dubliniensis* (n = 1, 3.6%), *C. tropicalis* (n = 3, 10.7%), *C. krusei* (n = 2, 7.1%), *C. glabrata* (n = 4, 14.3%), and *C. parapsilosis* (n = 2, 7.1%) (Table 2). The prevalence of nasal candidal carriage and the frequency of isolation of different

Candida species is presented in Table 2. *C. albicans and C. glabrata* were the most frequently recovered *Candida* species from the subjects, accounting for 17.9 percent each. The percentages of occurrence of other *Candida* spp. in the nasal cavities, in decreasing order, were as follows: *C. krusei* (n = 3, 10.7%) > *C. tropicalis* (n = 2, 7.1%) > *C. dubliniensis* (n = 1, 3.6%) (Table 2). Out of the 35 *Candida* spp. obtained, 19 (54.3%), 10 (28.6%), and 7 (20.0%) were positive for haemolysin, phospholipase, and amylase production, respectively. *C. albicans* (n = 3), *C. tropicalis* (n = 1), and *C. glabrata* (n = 2), were lipase producers; between 60% and 80% of *C. albicans* and *C. krusei* were haemolysin producers, while 50% of *C. parapsilosis* were phosphokinase-producing strains (Table 3). The percentage occurrence of caseinase-, gelatinase-, and biofilm-producing *Candida* spp. was 25.7%, 31.4%, and 20.0%, respectively. The candida isolates showing coagulase formation were *C. albic*ans (n = 2), *C. glabrata* (n = 1), and *C. tropicalis* (n = 1).

Itraconazole at a concentration of 10 mg/mL inhibited 100% of the tested *Candida* spp., with the highest mean zone of growth inhibition of 17.0 ± 1.0 mm (Table 4). The results showed that Itraconazole inhibited > 90% of *Candida* spp. (with the exception of *C. albicans* ST40 and *C. tropicalis* CT-O7) at a concentration of 5.0 mg/ mL, while all the *C. tropicalis* (CT-O7, CT-O14, and CT-N16) and *C. parapsilosis (*CP-O12 and CP-O21) displayed resistance to the growth inhibition of Itraconazole at a concentration of 2.5 mg/mL. The highest and lowest inhibition zone was 17.0 ± 1.0 mm and 8.6 ± 0.1 mm, respectively. The MIC values of Itraconazole ranged from 1.25 µg/mL for *C. albicans CA-O1, C. krusei* CK-O27, and *C. glabrata* CG-N3 to > 10 µg/mL for *C. albicans* CA-O25 and *C. tropicalis* CT-O7, the highest and lowest MFC obtained was 20 mg/mL and 2.5 mg/mL, respectively, while The MFC/MIC ratio ranged between 1 and 4 (Table 4).

The regression values of Itraconazole and Zone of Inhibitions (ZIDs) as exhibited by the *Candida* spp. ranged between 0.6554 to 1.0 (Table 5). The relationship between concentrations of Itraconazole and ZIDs as exhibited by *C. albicans* (CA-01; CA-O25 and CA-N19), *C. tropicalis* (CT-N16, CT-O7), *C. dubliniensis* (CD-N5), *C. parapsilosis* (CP-O12) and *C. glabrata* (CG-O19) are shown in Figures 1-8.

Table 1: Morphological Characteristics, Carbohydrate Fermentation and Enzymatic Tests of *Candida* spp.

		Growth at										Carbohydrate Fermentation Tests										Probable *Candida* spp
CHROM Agar™	Shape	35°C	45°C	Germ Tube	Nature of hyphae	Pseudo-hyphae	α-amylase	β-galactosidase	β-NAG	β-Glucuronidase	Urease	Inositol	Cellobiose	Glucose	Raffinose	Xylose	Trehalose	Maltose	Galactose	Sucrose	Lactose	
Blue-green	Oval	+	+	+	Septate	+	+	-	+	-	-	-	-	+	-	+	+	+	+	+	-	*Candida albicans*
Dark-green	Oval	+	-	+	Septate	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	*Candida dubliniensis*
Dark-blue	Oval	+	-	-	Septate	+	+	-	-	-	-	-	+	+	-	+	+	+	+	+	-	*Candida tropicalis*
Purple	Oval	+	-	-	Septate	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	*Candida krusei*
White	Oval	+	-	-	Septate	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	*Candida glabrata*
Pink	Oval	+	-	-	Septate	+	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	*Candida parapsilosis*

Keys: +: Positive; - : Negative; β-NAG: N-acetyl-β-glucosaminidase

Table 2: Percentage Occurrence of Oral and Nasal *Candida* spp

Candida spp.	Oral Cavities (n: 28)		Nasal Cavities (n: 28)		Total (n: 56) No. (%)
	No. of Occurrence	% of Occurrence	No. of Occurrence	% of Occurrence	
C. albicans	7	25.0	5	17.9	12 (21.4)
C. dubliniensis	1	3.6	1	3.6	2 (3.6)
C. tropicalis	3	10.7	2	7.1	5 (8.9)
C. krusei	2	7.1	3	10.7	5 (8.9)
C. glabrata	4	14.3	5	17.9	9 (16.1)
C. parapsilosis	2	7.1	0	0.0	2 (3.6)
Total No. (%)	19	67.9	16	57.1	35 (62.5)

Table 3: Virulence Markers of Oral and Nasal *Candida* spp

Candida spp.	No of Isolates	Virulence Markers/No. (%)							
		Haemolysin	Phospholipase	Amylase	Lipase	Caseinase	Gelatinase	Coagulase	Biofilm
C. albicans	12	8 (66.7)	3 (25.0)	1 (8.3)	3 (25.0)	3 (25.0)	4 (33.3)	2 (16.7)	3 (25.0)
C. dubliniensis	2	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)
C. tropicalis	5	2 (40.0)	2 (40.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (40.0)	1 (20.0)	1 (20.0)
C. krusei	5	3 (60.0)	1 (20.0)	2 (40.0)	0 (0.0)	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)
C. glabrata	9	4 (44.4)	3 (33.3)	3 (33.3)	2 (22.2)	2 (22.2)	2 (22.2)	1 (11.1)	3 (33.3)
C. parapsilosis	2	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)
Total	35	19 (54.3)	10 (28.6)	7 (20.0)	6 (17.1)	9 (25.7)	11 (31.4)	4 (11.4)	7 (20.0)

Table 4: Susceptibility of Oral and Nasal *Candida* spp. to Itraconazole

Candida spp.	Code	Mean Zone of Inhibition (mm ± S.D)			DMSO	µgmL^{-1}		Ratio MFC/MIC
		2.5 mgmL^{-1}	5 mgmL^{-1}	10 mgmL^{-1}		MIC	MFC	
C. albicans	CA-O1	12.4 ± 1.2^{a}	14.5 ± 1.0^{b}	15.9 ± 1.1^{b}	NZ	1.25	2.5	2
	CA-O25	NZ	NZ	9.7 ± 0.2^{a}	NZ	10	20	2
	CA-O11	10.9 ± 0.2^{a}	11.3 ± 1.2^{a}	12.1 ± 0.0^{a}	NZ	2.5	5	2
	CA-N9	10.0 ± 1.0^{a}	10.5 ± 0.0^{a}	12.0 ± 1.0^{a}	NZ	2.5	10	4
	CA-N19	NZ	9.2 ± 1.2^{a}	11.4 ± 0.2^{a}	NZ	5	20	4
	CA-N20	11.5 ± 0.5^{a}	12.8 ± 1.2^{a}	14.5 ± 0.5^{b}	NZ	2.5	5	2
C. dubliniensis	CD-O3	9.9 ± 0.2^{a}	11.5 ± 0.5^{a}	12.7 ± 0.1^{a}	NZ	2.5	10	4
	CD-N5	9.0 ± 0.0^{a}	12.0 ± 0.0^{a}	12.5 ± 0.5^{a}	NZ	2.5	10	4
C. tropicalis	CT-O7	NZ	NZ	10.0 ± 1.0^{a}	NZ	10	20	2
	CT-O14	NZ	11.3 ± 1.1^{a}	13.6 ± 1.0^{b}	NZ	5	10	2
	CT-N16	NZ	9.5 ± 0.5^{a}	11.9 ± 1.0^{a}	NZ	5	20	4
C. krusei	CK-O27	12.5 ± 0.5^{a}	15.0 ± 1.0^{b}	16.5 ± 1.5^{c}	NZ	1.25	2.5	2
	CK-N10	11.2 ± 0.1^{a}	12.5 ± 0.1^{a}	14.7 ± 0.3^{a}	NZ	2.5	5	2
	CK-N13	11.5 ± 0.5^{a}	13.8 ± 0.2^{b}	15.0 ± 0.0^{b}	NZ	2.5	2.5	1
C. glabrata	CG-O19	9.0 ± 0.5^{a}	11.0 ± 0.0^{a}	12.2 ± 0.1^{b}	NZ	2.5	10	4
	CG-O2	8.6 ± 0.1^{a}	10.5 ± 0.5^{a}	12.0 ± 0.0^{a}	NZ	2.5	10	4
	CG-N7	NZ	9.2 ± 0.2^{a}	10.7 ± 0.2^{a}	NZ	5	20	4
	CG-N3	12.1 ± 0.1^{a}	14.5 ± 1.0^{b}	17.0 ± 1.0^{c}	NZ	1.25	2.5	2
C. parapsilosis	CP-O12	NZ	8.5 ± 0.5^{a}	10.3 ± 1.2^{a}	NZ	5	20	4
	CP-O21	NZ	8.0 ± 0.0^{a}	10.0 ± 0.0^{a}	NZ	5	20	4

Keys: DMSO: Dimethylsulphoxide; NZ: No zone of Inhibition; MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungistatic Concentration; mm: Mean; S.D: Standard Deviation; each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 5: Regression Coefficient between Different Concentrations of Itraconazole and Inhibitory Zones Diameters Exhibited by *Candida* Isolates

Candida Isolates	Code	Regression (R^2)
C. albicans	CA-O1	0.9098
C. albicans	CA-O25	0.8929
C. albicans	CA-O11	1.0000
C. albicans	CA-N9	0.9918
C. albicans	CA-N9	0.7437
C. albicans	CA-N9	0.9872
C. glabrata	CG-O19	0.8929
C. glabrata	CG-O2	0.9349
C. glabrata	CG-N7	0.6961
C. glabrata	CG-N3	0.9685
C. krusei	CK-O27	0.8929
C. krusei	CK-N10	0.9982
C. krusei	CK-N13	0.8695
C. parapsilosis	CP-O12	0.7273
C. parapsilosis	CP-O21	0.7500
C. tropicalis	CT-O7	0.8929
C. tropicalis	CT-O14	0.7223
C. tropicalis	CT-N16	0.7515
C. dubliniensis	CD-O3	0.9276
C. dubliniensis	CD-N5	0.6985

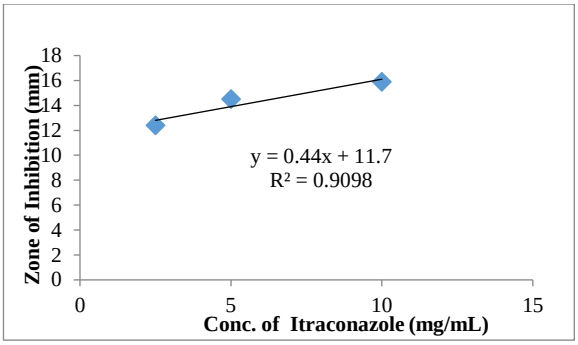

Figure 1: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CA-O1

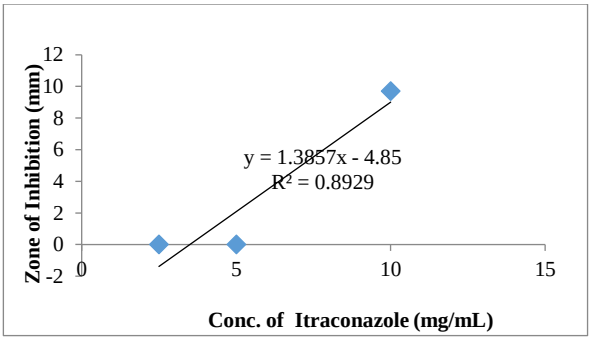

Figure 2: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CA-O25

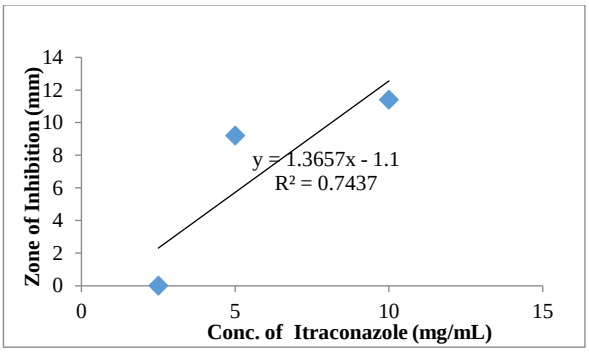

Figure 3: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CA-N19

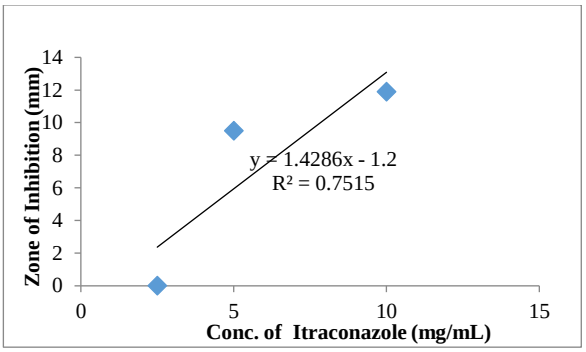

Figure 4: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CT-N16

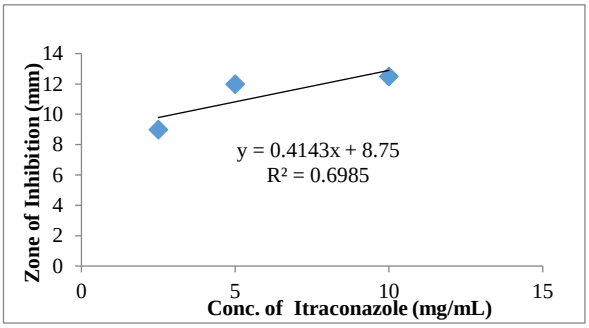

Figure 5: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CD-N5

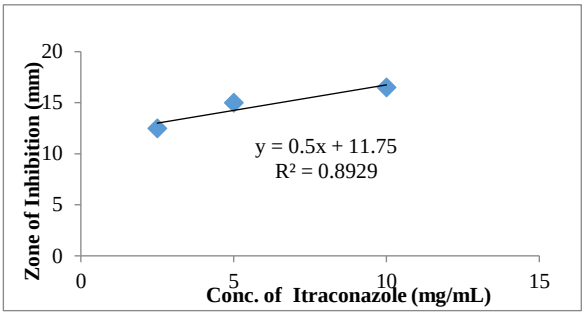

Figure 6: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CT-O7

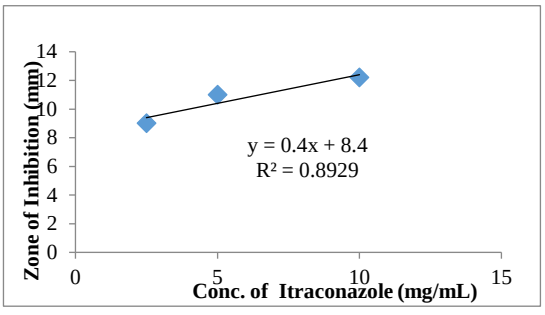

Figure 7: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CG-O19

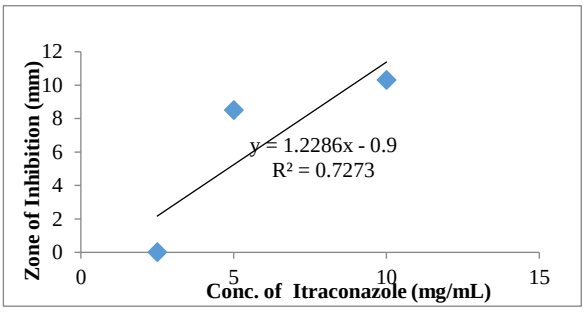

Figure 8: Relationship between Conc. of Itraconazole and Inhibitory Zones as exhibited by CP-O12

Discussion

Candida species are extensively found throughout the human population as both commensal organisms and occasional pathogens, and they exist harmlessly on mucosal surfaces. In this present study, Candida species isolated from the oral and nasal cavities were *C. albicans, C. dubliniensis, C. tropicalis, C. krusei, C. glabrata, and C. parapsilosis.* Isolation of *C. albicans, C. tropicalis, and C. parapsilosis from the oral cavities in our study substantiated the reports on* oral candida isolates among patients attending a tertiary hospital in southeast Nigeria *(*Nweze and Ogbonnaya, 2010). *The most common Candida species isolated from the oral cavities was C. albicans*, and this agrees with the findings of Sardi *et al.* (2013) in their study on the current epidemiology,

pathogenicity, and biofilm formation of Candida species in Brazil. Similarly, this finding is in agreement with Back-Brito *et al*. (2009) and Nweze and Ogbonnaya (2011), who found that the majority of yeast isolates from oral cavity swabs were *C. albicans,* and it was often recovered in association with other yeasts. *C. albicans* constituted the majority of the *Candida* spp. isolated from the oral cavity due to the possession of multiple adhesins and the secretion of extracellular hydrolytic enzymes. *C. glabrata was the second most common Candida spp. obtained from the oral cavities in our study, and this agrees with* Houang *et al.* (1997). In our study, the prevalence of *C. tropicalis* was 10.7%, and this value is lower than the 16% reported by Paula *et al.* (1990) and the 20.42% reported by Martins *et al.* (2002) in their studies on the presence of *Candida* spp. in the human oral cavity.

The percentage occurrence of Candidal isolates in nasal cavities in our study was 62.5%, and this value was higher than the 23% reported in Argentina (Nastri *et al*., 2012). Isolation of *C. albicans, C. tropicalis, and C. parapsilosis from the nasal cavities in our study corroborated the finding of Badiee et al.* (2017) in their study on the antifungal susceptibility testing of *Candida* spp. from the patients admitted to ten university hospitals in Iran. The nasal carriage of *C. albicans* (21.4%), *C. dubliniensis* (3.6%), and *C. krusei* (8.9%) in this study was higher than that of *C. albicans* (19%), *C. dubliniensis* (3%), and *C. krusei* (1%) reported by Nastri *et al*. (2012) on the nasal carriage of *Candida* species in Argentina.

The extracellular hydrolytic enzymes, which act synergistically under favourable conditions, contribute to the pathogenicity of *Candida* spp. (Silva *et al*., 2011) and are vital for the establishment of infections (Mba and Nweze, 2019). The production of these enzymes differentiates virulent, invasive strains from non-invasive strains. The occurrence of haemolysin, lipase, caseinase, and gelatinase-producing *Candida* spp. in this study corroborated the reports of Ying and Chunyang (2012) and Rossini *et al*. (2013). The haemolysin assists the isolates to lyse the host's erythrocytes and strips iron from haemoglobin molecules (Manns *et al*., 1994). In our study, 25.0% of the *C. albicans* were phospholipase-producing strains, and this value is lower than the 60% reported by Butola *et al.* (2015) in their study on virulence factors in clinical isolates of *Candida* spp. A low percentage (17.1%) of lipase-producing *Candida* spp were obtained from the oral and nasal cavities and this contradicts the high percentage (80%) of lipase-producing *Candida* spp previously reported by Pandey *et al*. (2018) and Akinjogunla *et al.* (2018). The non-production of lipase by *C. dubliniensis, C. krusei,* and *C. parapsilosis* in this study may suggest that the enzyme is probably not a significant virulence attribute of these species. Similarly, the present study showed biofilm production to be 20% among *Candida* spp., and this substantiated the finding of Marak and Dhanashre (2018) in their study on antifungal susceptibility and biofilm production of *Candida* spp. isolated from clinical samples. The pathogenicity of *Candida* species has also been reported to be associated with its capacity to produce biofilm, which is an essential virulence determinant during oral candidiasis (Mohandas and Ballal, 2011).

Reports have shown that Itraconazole is an active antifungal drug against serious yeast infections, such as oral thrush and Candida esophagitis (McGinnis and Rinaldi, 1996). All the Candida isolates (100%) were sensitive to Itraconazole at a concentration of 10 mg/mL, with the highest mean zone of growth inhibition of 17.0 ± 1.0 mm. The high sensitivity of *Candida* spp. to Itraconazole in our study contradicts the findings of Badiee and Alborzi (2011) in their studies on the susceptibility of clinical Candida species isolates to antifungal agents in southern Iran. The susceptibility of *C. tropicalis* and *C. parapsilosis* to Itraconazole at a concentration of 5 mg/mL in this study was low, as exhibited by the zone of inhibition, and comparable with the susceptibility rates reported in Nigeria (Akinjogunla *et al*., 2017). The discs containing 2.5 mg/mL showed the lowest (mean ± S.D) inhibition zones, while the discs containing 10 mg/mL of Itraconazole showed the highest zones of inhibition against the *Candida* spp.

CONCLUSION

The findings also pointed out that the higher the concentrations of the Itraconazole, the higher the sensitivities of the *Candid*a spp. to the Itraconazole. Consequently, this study has provided and updated data on the prevalence of *Candida* spp. in the mucosal surface and also revealed their susceptibility to Itraconazole.

ACKNOWLEDGMENTS

The authors would like to thank all the participants and the laboratory staff of the Department of Microbiology, University of Uyo, Uyo, Nigeria, for their technical support towards the successful completion of this work.

REFERENCES

Akinjogunla, O. J. and Eghafona, N.O. (2012). Mycological investigation in patients with AOM. *Scientific Journal of Microbiology* 1(1):19-26.

Akinjogunla, O. J., Divine-Anthony, O. Akpan, M.M. and Ogbonna, F.C. (2019). Virulence markers and antifungal susceptibility of vaginal yeast isolates from contraceptive and non-contraceptive users in Uyo, Nigeria. *Journal of Experimental Research*, 7(1): 33- 40.

Akinjogunla, O. J., Divine-Anthony, O., Ajayi, A. O., Etukudo, I. U. and Etok, I. J. (2020). Candiduria in Patients with Diabetic Type 1 and 2: Sociodemographic and Risk factors, prevalence, virulence factors and antifungal susceptibility, *Journal of Pure and Applied Microbiology*, 14(2):1467-1478.

Akinjogunla, O. J., Umo, A. N., Alozie, M. F., Oshosanya, G.O., and Saturday, G. I. (2021). Antibacterial potentiality and time kill kinetics of amlodipine, thioridazine and promethazine against pathogenic bacterial isolates from clinical samples. *African Journal of Clinical and Experimental Microbiology*, 22 (3): 397 - 406.

Akinjogunla, O.J., Ajayi, A.O. and Nkanga, I.E. (2018). Characterization, in-vitro detection of extracellular hydrolytic enzymes and antifungal susceptibility of faecal *Candida* isolates from diarrhoeal patients. *Nigerian Journal of Pharmaceutical and Applied Science Research.* 7:8-14.

Akinjogunla, O.J., Divine-Anthony, O. and Nwadialor, J. (2017). *Candida* isolates from high vaginal swabs of women of child bearing age: species distributions, virulence factors and antifungal susceptibility profile, *Nigerian Journal of Scientific Research*, 16 (4): 50-58.

Back-Brito, G.N., Mota, A.J., Vasconcellos, T.C., Querido, S.M.R., Jorge, A.O.C., Reis, A.S.M., Balducci, C.Y. and Koga-Ito, I. (2009). Frequency of *Candida* spp. in the oral cavity of Brazilian HIV-positive patients and correlation with CD4 cell counts and viral load. *Mycopathologia.* 167: 81-87.

Badiee, P. and Alborzi, A. (2011). Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: a five year study. *Iranian Journal Microbiology* 3(4):183–188.

Badiee, P., Badali, H. and Boekhout, T. (2017). Antifungal susceptibility testing of Candida species isolated from the immunocompromised patients admitted to ten university hospitals in Iran: comparison of colonizing and infecting isolates. *BMC Infectious Diseases* 17: 727.730.

Barnett, H.L. and Hunter, B.B. (1987). *Illustrated Genera of Imperfect Fungi*. 4th edn, McMillian, New York. 1987. 218 p.

Butola, R., Agwan, V., Thakuria, B. and Madan, M. (2015). A comparative study of virulence factors in clinical isolates of *Candida* species. *International Journal of Current Microbiology and Applied Sciences*. 4:716-722.

Calderone, R.A. (2002). Introduction and historical perspectives. *Candida and Candidiasis* (Calderone R., ed), ASM Press, Washington, DC. pp. 15–25.

Clinical Laboratory Standards Institute (2015). Method for antifungal disk diffusion Susceptibility testing of Yeasts; Approved Guideline-(4 Ed). CLSI document M44-A2. Clinical Laboratory Standard Institute, Wayne: Pennsylvania.

DaCosta, K.R., Ferreira, J.C., Komesu, M.C. and Candido, R.C. (2009). *Candida albicans* and *Candida tropicalis* in oral candidiasis: quantitative analysis, exoenzyme activity, and antifungal drug sensitivity. Mycopathologia, 167(2): 1-6.

Deorukhkar, S.C., Santosh, S. and Stephen M. (2014). Non-*albicans Candida* Infection: An Emerging Threat. *Interdisciplinary Perspectives on Infectious Diseases.* Article ID 615958.

Houang, E.T.S., Chu, K.C., Koehler, A.P. and Cheng, A.F.B. (1997). Use of CHROMagar Candida for genital specimens in the diagnostic laboratory. *Clinical Pathology.* 50: 563-565.

Manns, J.M., Mosser, D.M. and Buckley, H.R. (1994). Production of a haemolytic factor by *Candida albicans*. *Infection and Immunology*, 62: 5154–5156.

Marak, M.B and Dhanashree, B. (2018). Antifungal susceptibility and biofilm production of *candida* spp. isolated from clinical samples. *International Journal of Microbiology*. 1-5. Article ID 7495218.

Martins, C.A.D., Koga-Ito, C.Y. and Jorge, A.O.C. (2002). Presence of *Staphylococcus* spp. and *Candida* spp. in the human oral cavity. *Brazilian Journal of Microbiology*. 33:236-240.

Mba, I.E. and Nweze, E.I. (2019). Antifungal resistance profile and enzymatic activity of *Candida* species recovered from human and animal samples. *Bio-Research*. 17 (1); 1044-1055.

McGinnis, M.R. and Rinaldi, M.G. (1996). Antifungal drugs: mechanisms of action, drug resistance, susceptibility testing and assays of activity in biological fluids. In: Antibiotics in laboratory medicine. Lorain V, editor. Baltimore: Williams & Wilkins, pp. 176-211.

Mohandas, V. and Ballal, M. (2011). Distribution of Candida species in different clinical samples and their virulence: biofilm formation, proteinase and phospholipase production: a study on hospitalized patients in southern India. *Journal of Global Infectious Diseases*. 3(1):4-8.

Nastri, M.L., Hecht, P., Nastri, N., Jewtuchowicz, V., Cuesta, A., Estrugo, J., Gualtieri, A., Sordelli, D.O. and Rosa, A. (2012). Nasal carriage of Staphylococcus aureus and Candida species in immunocompetent individuals. *Acta Odontol Latinoam*. 25(3):324-329.

Nweze, E.I. and Ogbonnaya, U.L. (2011). Oral *Candida* isolates among HIV-infected subjects in Nigeria. Journal of Microbiology, Immunology and Infection. 44:172-177.

Okon, M. U., Akinjogunla, O. J., Akang, I.A., Akaka, B. C. and Umoh, M.I. (2020). Logarithmic and percentage reductions of bacterial isolates in *G. paradoxa treated* with *C. aurantifolia* and sodium chloride: public health implication and nutritional assessment. *FUDMA Journal of Science*, 4 (3): 406 - 415.

Pandey, N., Gupta, M.K. and Tilak, R. (2018). Extracellular hydrolytic enzyme activities of the different *Candida* spp. isolated from the blood of the intensive care unit-admitted patients. *Journal of Laboratory Physicians*. 10:4.

Paula, C.R., Sampaio, M.C., Birman, E.G. and Siqueira, A.M. (1990). Oral yeasts in patients with cancer of the mouth, before and during radiotherapy. *Mycopathologia*, 112: 119-124.

Pfaller, M.A and Diekema, D.J. (2012). Progress in antifungal susceptibility testing of *Candida* spp by use of clinical and laboratory standards institute broth microdilution methods, 2010 to 2012. *Journal of Clinical Microbiology.* 50(9):2846–2856.

Rafat, Z., Hashemi, S.J. and Ahamdikia, K. (2017). Study of skin and nail *Candida* species as a normal flora based on age groups in healthy persons in Tehran-Iran. *Journal of Medical Mycology.* 27(4): 501–505.

Rossoni, R.D., Barbosa, J.O., Vilela, S.F., Jorge, A.O. and Junqueira, J.C. (2013). Comparison of the hemolytic activity between *C. albic*ans and non-albicans Candida species. *Brazilian Oral* Research. 27(6):484-489.

Samaranayake, L.P. and MacFarlane, T.W. (1990). Oral Candidiasis. *Wright- Butterworth*, London, UK. pp 122-134.

Sardi, J.C.O., Scorzoni, L.T., Bernardi, A.M., Fusco-Almeida, M. and Giannini, M.J.S. (2013). Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology*. 62(Pt 1):10-24.

Schaller, M., Borelli, C., Korting, H.C. and Hube, B. (2005). Hydrolytic enzymes as virulence factors of *Candida albicans*. *Mycoses.* 48:365-377.

Silva, S., Negri, M., Henriques, M., Oliveira, R., David W.W. and Azeredo, J. (2011). Adherence and biofilm formation of

non-*Candida albicans* species. *Trends in Microbiology*, 19(5): 241-247.

Sun, J., Cheng, Q. C., Lafleur, M.D. and Qi1, Q. (2009). Fluconazole susceptibility and genotypic heterogeneity of oral Candida albicans colonies from the patients with cancer receiving chemotherapy in China. *International Journal* of *Oral Science*. 1(3): 156–162

Tsang, C.S., Chu, F.C., Leung, W.K., Jin, L.J., Samaranayake, L.P. and Siu, S.C. (2007). Phospholipase, proteinase and haemolytic activities of Candida albicans isolated from oral cavities of patients with type 2 diabetes mellitus. *Journal of Medical Microbiology*. 56(10): 1393–1398.

Ying, S. and Chunyang, L. (2012). Correlation between phospholipase of *Candida albicans* and resistance to fluconazole. *Mycoses*, 55(1):50–55.