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Diagnosis and Epidemiology of Urinary *Candida* Species in HIV-Positive Patients in a Nigerian Reference Medical Centre

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Abstract

Fungi thrive in hospitalized patients with underlying diseases. The urine samples of, one hundred and eight (108) human immunodeficiency virus (HIV)-positive patients currently on anti-retrovirals at the Federal Medical Centre Jalingo, Nigeria, were cultured on Sabouraud Dextrose Agar to isolate and investigate the occurrence, distribution, morphological, biochemical and genomic characters of *Candida* species. The prevalence of fungi was 62.0%. It was higher in females (68.0%) than in males (48.5%). All (100.0%) HIV-positive patients older than 74 years were infected while those between 55 and 64 years were least (33.3%) infected. Culturally, 43.3% of the fungal isolates had characteristic growth of *Candida* species while 56.7% had none. Microscopically, of the 29 *Candida* isolates, 79.3% had germ tubes typical of *Candida albicans* while 20.7% showed no hyphal extension (non-*albicans* species). Yeasts identified as *C. albicans* were subjected to molecular confirmation due to their close phenotypic relationship with other *Candida* species using the BLAST search. The similarity between the sequence queried and the biological sequences within the National Center for Biotechnology Information (NCBI) database that speciated the *Candida* isolated from the HIV-positive patients was approximately 100%. The HIV-positive patients that were between 25 and 34 years accounted for the highest prevalence (34.8%) of *Candida albicans* while none (0%) of those older than 74 years were infected. Infection rate was higher in females (69.6%) than in males (30.4%). Pregnant females were more (62.5%) infected than non-pregnant females (18.8%). Prevalence was least (18.8%) and equal in non-pregnant females and lactating but non-pregnant females. The effectiveness of the BLAST search, as a diagnostic tool in identifying *C. albicans* in HIV-infected individuals helped establish an association between *C. albicans* and HIV infections in patients currently receiving anti-retroviral medication, the distribution of which could possibly determine the clinical manifestations and management of infected patients.

Keywords: PCR, BLAST, Diagnosis, Prevalence, *Candida*, HIV, Anti-Retrovirals

Introduction

Candidiasis an opportunistic fungal infection caused by *Candida* species, can be transmitted in several ways including unprotected sexual activities. *Candida* urinary tract infection (UTI) is contemplated in patients with predisposing factors and those who are symptomatic of UTI and candidaemia (Qadir and Asif, 2019) [1]. The increased frequency of occurrence may possibly be due to their adaptability in many environments, a primary pathogenicity trait underlying their infection potential (Pappas *et al*., 2018) [2]. In recent years, *Candida* infections are becoming more prevalent and that, individuals with impaired immune systems such as those infected with HIV, are at risk for deep tissue and bloodstream infections that can progress and pose a threat to life (Gnat *et al*., 2021; Garbee *et al*., 2017; Makanjuola *et al*., 2018) [3, 4, 5].

Candidiasis in both women and men results in several pathologies including symptoms of vaginal and penile soreness, itching or burning sensations. Furthermore, women are more susceptible to candidiasis during pregnancy thereby, putting the newborn at risk of infection at childbirth (Rasti *et. al*., 2014; Umeh *et. al*., 2011; Nwadioha *et. al*., 2011) [6, 7, 8]. Consequently, in order to avoid complications especially in the immunocompromised, as a result of this co-infection, it is essential to quickly identify yeasts at the species level for accurate diagnosis and effective antifungal therapy (Jain *et. al*., 2012; Kim and Brehm-Stecher, 2015) [9, 10].

The epidemiological pattern exhibited by most fungi may be dependent on factors such as malnutrition and immune status of an individual as well as the influence of increased antifungal usage, the latter, which has been implicated with the development of resistance (Magill *et. al*., 2006; Marie and White, 2009) [11, 12].

Conventionally, the differential diagnosis of candiduria is by urine culture while the germ tube, sugar assimilation and

fermentation tests are used to speciate *Candida* isolates. Newer methods like the Chromogenic (CHROM) agar, analytical profile index (API) and Vitek 2 identification systems can be used but are expensive (Matare *et al*., 2017; Sumitra and Maheshwari, 2014) [13, 14].

It is difficult to isolate and culturally identify fungal colonies due to the few differences in their colonial and morphological features. Thus, genomic DNA extraction and sequencing technologies such as BLAST are used to characterize and confirm their identity by sequencing the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) with universal primers, ITS-1 and ITS-4, to amplify the ITS target region (White *et al*., 1990; Altschul *et al*., 1997) [15, 16].

Materials and Methods

Study Area

This study area was located in Jalingo town, the urban and cosmopolitan capital of Taraba State, northeastern Nigeria. It lies in the Savannah-covered foothills of Shebshi Mountains and has an average temperature of 29^0C and humidity of 49 percent. It is connected by road with Yola and Wukari. As of November 2022, Jalingo has an estimated population of 581,000 inhabitants living in an area that is around 3,871 square kilometers with the most prominent tribes being Fulani and Mumuye. Christianity and Islam are the widely practiced religions in the area while the inhabitants are mainly farmers, marketers and civil servants.

Study Population and Sample Collection

Ethical clearance was obtained from the Ethical Committee of the Federal Medical Center, Jalingo in Taraba State. Thereafter, one hundred and eight (108) HIV-infected out-patients attending the Center, as part of the monitoring exercise of HIV progression and collection of free anti-retrovirals, were randomly selected for this study. Participation was purely on voluntary basis. Early morning mid-stream urine samples were collected in clean, sterile universal bottles and quickly transported to the Microbiology laboratory of Federal University Wukari, for microbiological analyses. Data was anonymously analysed and results expressed as percentages.

Inclusion and Exclusion Criteria

These were HIV-positive patients attending the Medical Centre who, consented to be part of the study, have resided in Taraba State for more than one year prior to sampling, were more than 15 years old and, with or without symptoms of genitourinary candidiasis. The exclusion criterion was HIV-negative patients attending the Medical Center were excluded from participating.

Media Preparation

Sabouraud Dextrose Agar was used as the medium for the cultivation of urinary fungi and it was prepared according to the manufacturer's instruction.

Culture, Isolation and Identification of Fungi

Urine samples were cultured on Sabouraud dextrose agar (SDA) containing chloramphenicol (1mg/ml) and incubated at 37^0C for 72 hours under aerobic conditions. Thereafter, the plates were observed for characteristic growth of *Candida* (white to cream colored colonies that are smooth and yeast-like in appearance). These colonies were sub-cultured on SDA to obtain pure colonies that were then Gram stained as described by Cheesbrough (2012) [17].

Germ Tube Test

This was used as a rapid tool for the identification of *C. albicans.* A pure colony of *Candida albicans* was harvested using a sterile wire loop, and inoculated into a sterile test tube containing 0.5ml of human serum. The culture was incubated aerobically at 37^0C for 3 hours. Subsequently, a drop of the yeast-serum liquid culture was placed on a clean microscope slide. A drop of lactophenol cotton blue stain was added to it before it was covered with a cover slip. The drop was examined microscopically, using both the x10 and x40 objective lenses of a microscope. The appearance of small sprouting tube-link outgrowths or filaments projecting from the cell surface confirmed the production of germ tubes.

Molecular Characterization of *Candida* Species

Conventional Polymerase Chain Reaction (PCR) was used to characterize the fungal isolates by sequencing the ITS regions of their nuclear DNA (rDNA). The target and 5' to 3' sequence of ITS-1 primer was ITS rDNA sequence and TCCGTAGGTGAACCTGCGG respectively while the target and 5' to 3' sequence of ITS-4 primer was ITS rDNA sequence and TCCTCCGCTTATTGATATGC respectively.

The ITS target regions were amplified using ITS-1 and ITS-4 (White *et al*., 1990) [15]. Genomic DNA was extracted from the samples using the Quick-DNA™ Miniprep Plus kit (Zymo Research, Catalogue No. D4068). Using the OneTaq@ Quick-Load 2x Master Mix (NEB, Catalogue No. M0486), the COI target region was amplified with the previously described ITS-1 and ITS-4 (Altschul *et al*., 1997) [16].

The PCR products were run on a gel and cleaned up enzymatically using the EXOSAP method. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrillantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample. BioEdit Sequence Alignment Editor version 7.2.5 was used to analyse the ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI).

Results

Table 1 shows the morphological characteristics of the isolated *Candida* spp. The variations in their colonial features were used in their identification. *Candida* spp. occurred as spherical or elongated cells in clusters.

The prevalence of fungi among the HIV-positive patients was 62.0% (Table 2). It was higher in female patients (68.0%) than in the male patients (48.5%). All the HIV-positive patients that were older than seventy-four years old had fungal infections (100.0%) while those within the 55-64 years age group had the least (33.3%) prevalence (Table 3).

Culturally, twenty-nine (43.3%) of the fungal isolates showed the characteristic growth of *Candida* species while thirty-eight (56.7%) did not manifest any morphology that was characteristic of *Candida* species (Table 4). Microscopically, of the 29 *Candida* isolates, 79.3% had

short slender, tube-like structures (germ tubes) typical of *Candida albicans* while 20.7% showed no hyphal extension from the yeast (non-*albicans* species) cell (Table 5).

Yeasts identified as *C. albicans* by culture, Gram and lactophenol cotton blue stain reactions and, germ tube formation, were subjected to PCR-BLAST confirmation due to their close phenotypic relationship with other *Candida* sp. using an algorithm that compares primary biological sequence information of sequences the nucleotides of DNA and calculates the statistical significance of matches. The BLAST search identified that the similarity between the sequence queried and the biological sequences within the NCBI database that speciated the *Candida* isolated from the HIV-positive patients was approximately 100% (Table 6).

The HIV-positive patients between 25 and 34 years of age accounted for the highest prevalence of *C. albicans* (34.8%) while none (0%) of those that were more than seventy-four years old were infected (Table 7). In Table 8, the prevalence of *C. albicans* was higher (69.6%) in the female than in the male patients (30.4%). Pregnant females were more (62.5%) infected than non-pregnant females (18.8%). Prevalence was least (18.8%) and equal in non-pregnant females and lactating but non-pregnant females (Table 9).

Table 1: Morphological characteristics of fungi isolates

Colony characteristics (macroscopy)	Microscopic appearance	Probable fungi isolate
Smooth, small sized, cream colored colonies	Spherical cells in clusters	*Candida* spp.
Grey colored, filamentous, colonies. Older cultures were brown colored	Large conidia head with hyphae	*Aspergillus* spp.
Smooth, irregular, cream colored colonies	Elongated or spherical cells in clusters	*Candida* spp.
White filamentous colonies. Older cultures were brown colored	Large conidial heads with split hyphae	*Aspergillus* spp.
Dark-greenish filamentous colonies	Unbranched and rough conidiophores	*Aspergillus* spp.

Table 2: Sex-related prevalence of fungi among the HIV-infected patients

Gender	Number examined	Number infected	Prevalence (%)
Male	33	16	48.5
Female	75	51	68.0
Total	108	67	62.0

Table 3: Age-related prevalence of fungi in HIV-positive patients

Age group	Number examined	Number infected	Prevalence (%)
15-24	15	8	53.3
25-34	34	27	79.4
35-44	31	16	51.6
45-54	11	5	45.5
55-64	6	2	33.3
65-74	7	5	71.4
≥ 75	4	4	100
Total	108	67	62.0

Table 4: Distribution of fungi in HIV-positive patients using SDA cultures (n = 108)

Type of isolate	Number of patients infected	Percentage (%)
Candida species	29	43.3
Mould	38	56.7
Total	67	62.0

Table 5: Distribution of candiduria *Candida albicans* using the germ tube test (n = 29)

Type of isolate	Number of patients	Percentage (%)
Candida albicans	23	79.3
Non-*Candida albicans*	6	20.7
Total	29	26.9

Table 6: BLAST confirmation of *Candida albicans* in urine samples of HIV-positive patients

Sample code	Percentage ID	GenBank accession number	Predicted organism
Candida spp.	97.5	XR_002086443.1	*Candida albicans*
Candida spp.	100	XR_0022086443.1	*Candida albicans*

Table 7: Age-related prevalence of *Candida albicans* in HIV-positive patients (n=23)

Age group	Number infected	Prevalence (%)
15-24	6	26.0
25-34	8	34.8
35-44	4	17.4
45-54	2	8.70
55-64	1	4.35
65-74	2	8.70
≥ 75	0	0
Total	23	100

Table 8: Gender-related prevalence of *Candida albicans* in HIV-infected patients (n=23)

Gender	Number infected	Prevalence (%)
Male	7	30.4
Female	16	69.6
Total	23	100

Table 9: Distribution of *Candida albicans* in HIV-infected female patients (n=16)

Status of HIV-positive female	Nos. infected *C. albicans*	Prevalence (%)
Pregnant	10	62.5
Lactating but non-pregnant	3	18.8
Non-pregnant	3	18.8
Total	16	100

Discussion

Opportunistic fungal infections account for a significant amount of morbidity associated with HIV disease. It is considered as an important marker of immune suppression and may be the initial manifestation of the disease in about 10% of HIV-infected adults (Aberg *et al*., 2014) [18].

In this study, the isolates that were inoculated on SDA medium showed the characteristic growth of *Candida* species. However, the difficulty speciating them based on their cultural features alone was overcome by the successful identification of isolates from cultures suspected to be *C. albicans* using the germ tube formation (Matare *et al*., 2017) [13]. Comparatively, the accurate prediction and confirmation of the similarity between the sequence of *Candida* spp. isolated from the HIV-positive patients and the biological sequences within the NCBI database, showed that the PCR-BLAST search of nucleic acid base sequence is not only important as a tool in the taxonomic differentiation of *Candida* spp., but also in the determination of the epidemiology of *C. albicans.* The observed age-related disparities in the prevalence and distribution pattern in this

present study supports the previous reports that linked candidiasis to age with the highest rates recorded in younger adults (Anwar *et al*., 2012; Mbakwem-Aniebo *et al*., 2020; Holzer *et al*., 2017) [1, 19, 20, 21]. Obviously**,** in this investigation young adults between 15 and 24 years represent the most active components of the population that are directly linked to the risk factors of *C. albicans* candidiasis. The female vagina harbours naturally occurring microorganisms. However, acts of coitus, indiscriminate antimicrobial use, contraceptive use, pregnancy and other associated factors can exacerbate commensal overgrowth and cause vaginitis, a condition that could predispose females to candidiasis. Significantly higher susceptibilities to candidiasis in pregnancy occur because of hormonal changes during pregnancy. The colonization of the vagina with *Candida* spp. during pregnancy has been associated with impaired pregnancy outcomes and is more harmful in the second trimester of pregnancy (Holzer *et al.*, 2017) [21]. Candiduria is common in the natural life of most females due to reasons previously adduced herein. Thus, it is not surprising to record higher rates in females than males.

Conclusion

The BLAST search was effective in identifying *C. albicans* in HIV positives on anti-retrovirals, the distribution of which could determine the clinical manifestations and management of patients.

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