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Species Diversity of *Candida* from Hospital Environment in Plateau State, Nigeria

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Abstract: In the past few years the role the hospital environment in the transmission of infections has increased leading to the increase in the incidence of morbidity and mortality associated with *Candida* infections. The hospital environment can serve as an alternative niche for the presence of opportunistic pathogens of public health concern. This study sought to use molecular approach to study the prevalence of *Candida* from hospital environments in Jos, Plateau State, Nigeria. Samples were collected from different hospital environments and cultured on Chromagar *Candida* media. Taqman PCR was then used to characterize the isolates, the ability of *Candida* to form biofilm was evaluated. In this study, *Candida parapsilosis* with 31.25% was the most prevalent *Candida* followed by *Candida (Nakaseomyces) glabrata* and *Candida krusei* with prevalence of 28.13%. The ability to form biofilms was evaluated for these environmental isolates, and it was observed that *C. parapsilosis* biofilm formation correlated with high prevalence with *C. parapsilosis* having high biofilm formation (20/64) followed by *C. glabrata* and *C. krusei* (18/64) each Environmental sampling has the potential to improve our knowledge about yeast biodiversity and the evolution of pathogenic yeasts with clinical relevance. This study is the first in Nigeria to study to characterize *Candida* from hospital environments in Jos, Plateau state Nigeria.

Keywords: Hospital Environments, *Candida*, Taqman PCR, Biofilm

1. Introduction

Fungal nosocomial infections have increased in frequency in the past few years [1] and the Hospital environment has played a major role in the transmission of nosocomial *Candida infections [2, 3]*. The frequency of hospital acquired fungal infections have increased in recent times with concomitant increases in morbidity and mortality. The recent outbreak of multi-resistant strain of *C. auris [4]* a pathogen with no known ecology but has been isolated from hospital environment that has been an increase need to look at our

hospital environment to find out how safe they are for patients and visitors. There is a shift away from *Candida albicans* as a major causes of candidemia to non-albicans *Candida* (NAC). Cases of NAC reported from hospital environment include *C. auris* [3, 5, 6], *C. parapsilosis [2, 7]*, *C. glabrata [8]*. In a recent study by Opulente *et al.* [9] they showed that these common pathogenic yeast have ecological niches outside the clinical settings and may persist in alternative niches with ecology that may be more

complicated than is currently assumed. There is a dogma that the infective strains of the human normal gut flora (*C. albicans* and *C. glabrata*, and the skin for *C. parapsilosis)* comes from the patients endogenous flora, however the isolation of these pathogens from alternative niches tends to challenge this dogma [10]

The hospital environment harbor a lot of other pathogens, these pathogens may be transmitted to individuals in many ways ranging from hands of healthcare providers [7], catheters [11] and contact with hospital environments like beds [12]. In Nigeria, little or nothing is known about the epidemiology of fungal infections and the species distribution of *Candida* pathogens in the hospital environment. This study sought to use molecular approach to characterize isolates of *Candida* from hospital environment in Plateau State, Nigeria.

2. Materials and Methods

Yeast samples were obtained from different hospital environments (laboratory, wards, doors handle, bench surfaces and hands of health workers and air) sources in a secondary care hospital, and only those that contained yeasts were maintained for further processing. Samples were collected from hospital environments, that is, seats used patients visiting the hospital doors handle, sheets. Environmental air samples were collected in 4-cm diameter petri dishes containing Sabouraud agar, which were exposed to the environment for 25 min.

Samples from surfaces were collected using sterile swabs sticks and were transported to the Microbiology Laboratory, Department of Microbiology of Plateau State University Bokkos within an hour after collection. Each swab was rolled onto Sabouraud dextrose agar (SDA) plate supplemented with chloramphenical (0.5g/L) and incubated at 37°C for 2 days.

Distinct yeast colonies from each culture plate were subcultured on HiCrome *Candida* differential media plates for the identification of Candida species. These plates were incubated at 37°C for 2 days. Identification was done by the specific colour of colonies on the media as described by the manufacturers. Subcultures were made from chromogenic agar plates onto Sabouraud dextrose agar slants and sterile water. Slants were examined for growth after 24-48 hours incubation at 37°C and stored. These slants served as stocks from which subcultures were made for further characterization of the isolates.

2.1. Identification of the Candida Species Using Taqman Probes

MagNA Pure Compact Nucleic Acid Isolation Kit I and MagNA Pure Compact (Roche, Germany) was used for nucleic acid isolation. Briefly, Colonies were obtained from pure culture on a Petri dish and put in to an eppendorf tube. Then 500μl lyticase lysis buffer (50 mM Tris (pH: 7.6), 1mM

EDTA (pH: 8.0), %2 2-merkaptoetanol, 10 U lyticase (Sigma) were added and incubated at 37°C for one hour. Five hundred-(500) μl supernatant was taken and put in to automatic nucleic acid isolation machine. The final volume was adjusted for 100 μl. Taqman probes (Table 1) were used for identification of the *Candida* species.

PCR Conditions. Light Cycler 2.0 (Roche, Germany) and Light Cycler Taqman Master (Roche, Germany) was used for real time PCR. It was used this PCR conditions: Initial denaturation is 10 min at 95°C; Amplification was performed 45 cycle (denaturation is 10 sec. at 95°C; annealing is 20 sec at 53°C; extension is 1 sec at 72°C (single)) and followed by cooling step was 30 sec at 40°C. Finally analyzes were performed by Light Cycler program. VITEK was used to confirm the presence of C. famata.

2.2. Biofilm Production

Biofilm production was detected by tube method described by Branchini et al (1994) [13], and evaluated using the method of Shin et at [14]. A loopful of organisms from sabouraud's Dextrose agar (SDA) plate was inoculated into Sabouraud's Dextrose Broth supplemented with glucose (final concentration 8%). Polystyrene microtiter plates were then incubated at 37°C for 24 hours after which the broth was aspirated out gently. The microtiter wells were then washed once with distilled water and then stained with 1% safranin. The microtiter plate was kept still for 7 minutes. Safranin was then removed, and the wells were examined for biofilm production. Biofilm production was read independently by two different observers. The adherent biofilm layer was scored visually as either negative or weak positive (+), or moderate positive (++) or strong positive (+++). All positive results, including weak or strong, were considered as positive.

3. Results and Discussion

There has been a recent increase in NAC associated infection in hospitalized patients [15]. These species have also shown reduced susceptibility to some antifungals and have posed a problem to clinicians. In this study, a high prevalence was observed for *C. parapsilosis* 31.25% followed by *C. glabarata* and *C. krusei* with a prevalence of 28.13% each (Table 1). The high prevalence of *C. parapsilosis* is consistent with most studies that have shown a high occurrence of this species in hospital environment and in association with Catheter tips [16]. *C. parapsilosis* accounts for a significant proportion of nosocomial infections with an increasing prevalence in hospital settings [2, 17]. It has been reported as a major cause of candidemia in paediatric units [18] with exogenous and environmental sources identified as the source of infection [2]. This study contrasts what was reported by Savastano el al. (2016) [8] in Brazil, in their study, they found a high occurrence of *C. albicans* followed by *C. glabrata* and *C. parapsilosis* in that order.

Table 1. *Taqman probes which used for identification of Candida species.*

Species Name	Tagman probes
Candida albicans	FAM-CATTGCTTGCGGCGGTA-TAMRA (0.2 M)
Candida tropicalis	FAM-GGCCACCACAATTTATTTCA-TAMRA (0.2 M)
Candida parapsilosis	FAM-GAAAGGCGGAGTATAAAC-TAMRA (0.2 M)
Candida glabrata	FAM-GTTTTACCAACTCGGTGTTGAT-TAMRA (0.2 M)

Different distribution have been reported through out the world, *C. albicans* and *C. glabrata* were the most frequently isolates from Denmark and the USA [19]. The distribution of the Candida isolates have changed in the last decased with the decrease in the proportion of *C. albicans* and with and increase in *C. paraspsilosis* and *C. glabrata* infections [20]. An increasing trend have been observed for candidemias caused by non-albicans *Candida,* in the US, *C. glabrata* accounts for one-third of the candidemias [21] followed by *C. parapsilosis* which accounts for 15% of all isolates [22]. In South Africa, *C. albicans* and *C. parapsilosis* are the dominant species found [23]. In Nigeria however, the diversity of *Candida* species implicated in candidemia is not known as well as the distribution of *Candida* genotypes from hospital environment is scarce. Our study is the first to look at occurrence of *Candida* species in hospital environment in Nigeria. According to our results, *C. tropicalis* and *C. famata* had the lowest rate of isolation, as also reported in another study [24]. Although a different isolation frequency has been reported for *C. tropicalis* in another study. Hallur, Mahapatra, Deb, Tripathy, Misra and Praharaj [25] reported a higher occurrence of *C. tropicalis*. *C. famata* (*Debaryomyces hansenii)* yeast commonly found in natural substrates and in various types of cheese [26], although it has been reported in human infections its occurrence is rare [27, 28]. It has been misidentified as *Pichia guilliermondii*, *P. caribbica*, *P. jadinii*, *D. hansenii*, *Candida palmioleophila*, *C. haemulonii* type II, and *Clavispora lusitaniae*. Suggesting therefore that *C. famata* may not be a human pathogen as thought [29].

Table 2. *Distribution of Candida species isolated from Hospital environments in Plateau state, Nigeria.*

Candida species	Catheter tips	Hospital (AIR)	Other Surfaces	Total
Candida parapsilosis	20	0.0	0.0	20 (31.25)
Candida tropicalis	4	1	2	5 (6.25)
Candida krusei	0.0	8	10	18 (28.13)
Candida glabrata	0.0	6	12	18 (28.13)
Candida famata	0.0	3	0.0	3 (4.69)
Total	4	18	24	64

The adherence capacity of diverse Candida species play a role in colonization and subsequent invasion of these *Candida* species as well as the perpetuation of infection caused by these various species [30]. Several factors can predispose a person to Candida colonization, these factors include gastrointestinal tract colonization by *Candida* species resulting from prolonged use of broad-spectrum antibacterial agents, disruption of the gastrointestinal mucosal surfaces by cytotoxic agents or hypotension, and neutropenia [31]. Central venous catheters (CVCs), however, appear to be the most common risk factor for the development of candidemia in patients without neutropenia or major immunodeficiencies [32]. Biofilms formation on prosthetic materials have been associated with enhanced virulence of *C. parapsislosis [13]*. All the *Candida* isolated obtained in this study produced biofilms. The rank order of biofilm production of these *Candida* isolates is shown in table 3. Considerable difference exist in the way different species formed biofilms.

Table 3. *Biofilm Production by some of the Candida isolates obtained in this study.*

Candida species	Strong Positive (+++)	Moderate Positive (++)	Weak Positive (+)	Weak or Negative (0/-)	Total
Candida parapsilosis	1	0.0	8	11	20
Candida tropicalis	0	1	1	3	5
Candida krusei	0.0	3	7	8	18
Candida glabrata	2	4	12	3	18
Candida famata	0.0	0.0	0.0	3	3
Total	3	8	28	28	64

4. Conclusion

This study has reported for the first time the species diversity of *Candida* isolates that are found in a hospital environment in Nigeria. This paper provides a baseline, whereby future studies have the potential to demonstrate a rise in non-albicans species and their possible role in pathogenesis. These would go a long way in stimulating research to finding out which species of *Candida* is more

prevalent in patients with candidemia in Nigeria and would help clinicians in the management of such cases.

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