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SHORT COMMUNICATION

MORPHOLOGICAL VARIATIONS IN COLONY AMONG CLINICAL *CANDIDA* STRAINS

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ABSTRACT

A study was undertaken to document the colony morphology (surface, shape, consistency, elevation and colour) in *Candida* strains. Samples were collected from symptomatic female patients in some cities in Nigeria. Microscopically-detected (direct mount wet preparation) *Candida* – positive samples (n=585) were used. The samples made up of high vaginal swabs (83%), endocervical swabs (14%) and urine (3%) were plated on Sabouraud Glucose Agar and incubated at 37^0C for 48 hours. The surface and consistency types were determined by touching each colony with an inoculation loop (4mm). The cultures yielded 595 isolates of *Candida*, made up of seven species. Three colony surfaces were identified – Wet (W), Dry Shiny (DS) and Dry Powdery (DP) - with the DS having the highest distribution (65.1%) and DP, the least distribution (12.7%). Also, there were three distinguishable colony consistencies – Creamy (C), Dry Hard (DH), and Dry Grannular (DG) - with distribution of 87, 12 and 1%, respectively. Most of the species showed raised colonies. There were species - specific and intra- specific variations in surface and consistency. Statistically ($p \leq 0.05$), some of the colony features were species-dependent while others were not. The results provide an all-encompassing information on the colony morphological diversity in *Candida* species which is valuable in the understanding of the biology of *Candida*.

Keywords: *Candida*, colony morphology, diversity, genitourinary specimens.

INTRODUCTION

Globally, *Candida* species are found in man as harmless commensals in the genitourinary tract and other sites (Alexopoulos, and Mims, 1979; Carlsen, 2001; Ahmad *et al*., 2004; Vidotto *et al*., 2003). The species may become pathogenic however, due to a disruption of the microflora balance (Carlsen, 2001; Yang *et al*., 2006).

The colony morphology exhibited by a microorganism (including fungi) is an important characteristic which can be used, to an appreciable extent, to distinguish such an organism from others (Taylor *et al.,* 1998). Fungal species show some degree of colony variation in surface and consistency (Lipperheide *et al.,* 2002). As a follow up to earlier reports on *Candida* cell morphology (Dede and Okungbowa, 2009; Okungbowa *et al*., 2009) this study was undertaken to document some of the different colony surface types and consistencies present in *Candida* species in order to contribute to the understanding of their diversity, an important factor in the control and eradication strategies for an offending organism such as *Candida.*

MATERIALS AND METHODS

High vaginal swabs (HVS), endocervical swabs (ECS) and urine was collected from symptomatic female patients attending hospitals and medical diagnostic laboratories in some cities in the southern part of Nigeria. A total of 585 *Candida* positive samples (detected by direct mount wet preparation) made up of HVS 480, ECS 22, Urine 15, were collected. Solid cultures of the samples were prepared using Sabouraud Glucose Agar (SIFIN, Berlin, Germany) and incubated at 37^OC for 48 hours.

Candida species were identified by the CHROM Agar (Houang *et al.,* 1997) and the API 20C System (Analy-tab Products, Plainview, USA) (Rex *et al.*, 1995) methods. The surface of each colony was touched lightly with an inoculation loop (4mm diameter) to determine its type of surface and consistency (Taylor *et al.*, 1998). Colonies were examined with a magnifying glass to determine shape and elevation while colours were distinguished following the descriptions of Leboffe and Pierce (1996) and Taylor *et al*. (1998).

RESULTS AND DISCUSSION

The isolates recovered from the samples were 595, represented by seven *Candida* species with a distribution pattern as shown in (Fig. 1). There were three distinct colony surface types, namely, wet (W) dry shiny (DS) and dry powdery (DP) (Table 1), with the DS type having the

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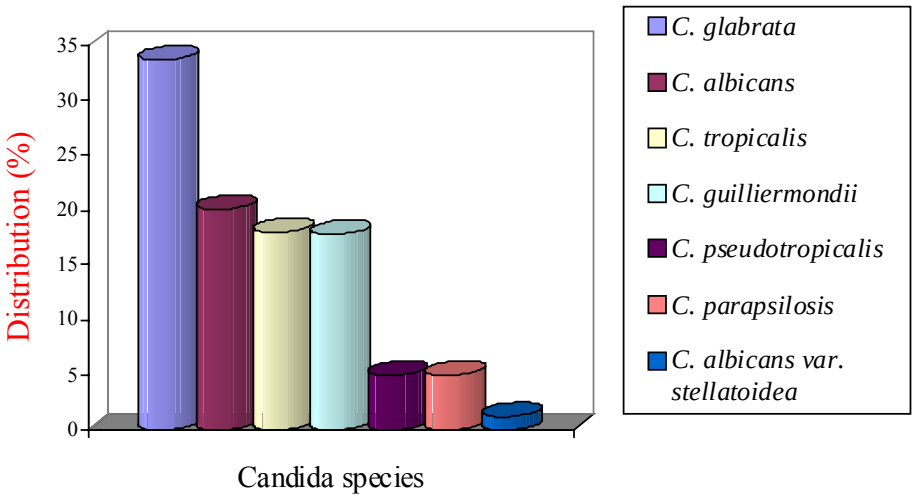

Fig. 1. Distribution of *Candida* species among the total isolates.

Table 1. Distribution of colony surface types among *Candida* species.

Colony Surface	Distribution among *Candida* species (%)							
	C. glab	*C. trop*	*C. guill*	*C. alb*	*C. pseu*	*C. para*	*C. alb. var. stell*	% Total*
Wet	43	-	-	31.4	100	-	-	21.9[a]
Dry Shiny	57	61	100	68.6	-	-	-	65.1[b]
	-	-	-	-	-	100	100	12.7[c]

*Values with different alphabets in superscripts along the same column are statistically different ($p \leq 0.05$).

highest frequency (65.1%) and the DP, the least (12.7%). In the distribution of surface types, 43% *C. glabrata,* 31.4% *C. albicans* and 100% *C. pseudotropicalis* exhibited the W type of surface. The DS surface was found in 57% *C. glabrata*, 61% *C. tropicalis*, 100% *C. guilliermondii* and 68.6% *C. albicans*. The DP surface was present in 39% *C. tropicalis*, 100% *C. parapsilosis* and 100% *C. stellatoidea*. Three colony consistency types were distinguished; these were creamy (C), dry hard (DH) and soft granular (SG), with the C type having the highest frequency of occurrence (Fig. 2). All isolates of *C. stellatoidea* had the SG consistency. The distribution of colony shape among the different species did not follow a particular pattern (Table 2). The colony shapes and their distribution in the total isolates were conical (14%), umbonate (41%), flat-topped (29%) and irregular (16%). Most species showed raised colonies (Table 3). Three colony colors were also observed, namely, off-white, brownish, and white (with 93, 3.5 and 3.0% distribution, respectively), see table 4.

Apart from several factors ranging from substrate composition and pH, and environmental factors which determine the surface and consistency of a fungal colony, the concept is also genetically determined (Rodgers and Beardall, 1999; Lipperheide *et al.*, 2002). Different colony surfaces and consistencies have been reported by Lipperheide *et al.* (2002). Colony shape is a basic distinguishing characteristic in the study of lower fungi (Taylor *et al.*, 1998). Colony colour (by means of chromogenic media) varies in *Candida* species (Houang *et al.*, 1997) and has been used to identify them both in antifungal susceptibility testing and virulence studies. Additionally, Rodgers and Beardall (1999) suggested a correlation between colony colour and virulence in *Candida* species. Several factors such as substrate composition, pH, temperature, among others, may be responsible for colour variation. In curtailing the menance of an offending organism such as *Candida*, its entire biology (including its adaptation to different ecological nitches) must be studied. Okungbowa *et al.* (2003)

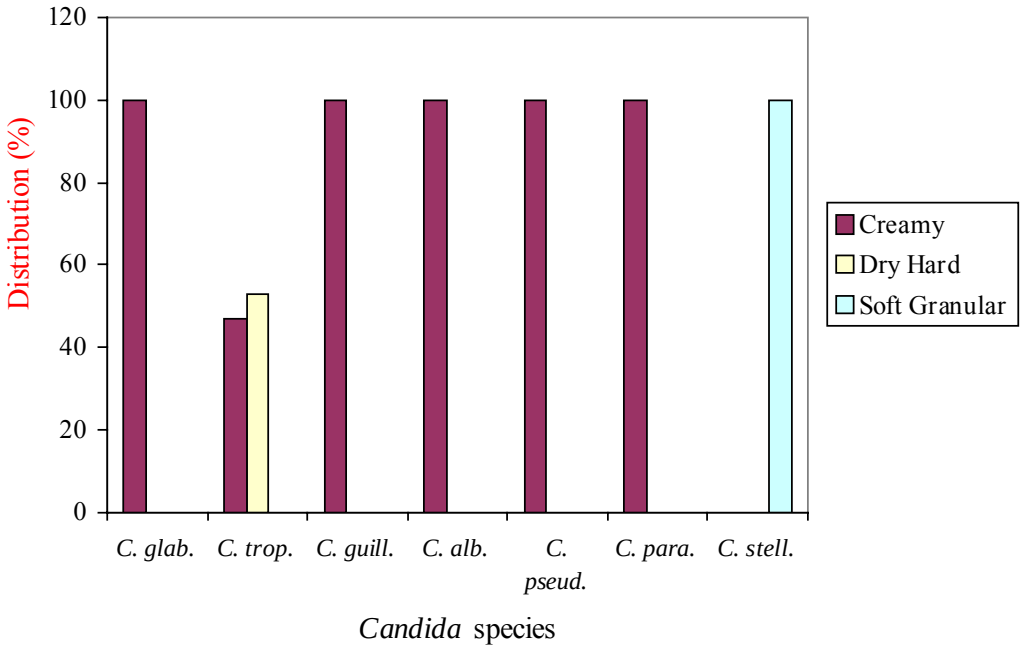

Fig. 2. Distribution (%) of colony consistency types among *Candida* species.

Table 2. Distribution of colony shapes among *Candida* species.

Type of colony shape	Distribution among *Candida* species (%)							
	C.glab.	*C. trop.*	*C. guill.*	*C. alb.*	*C. pseu.*	*C. para.*	*C.alb. var stell.*	%Total*
Conical	-	61	-	-	-	-	-	14[a]
Umbonate	100	40	-	10.5	-	-	100	41[b]
Flat-topped	-	-	100	-	100	100	-	29[c]
Irregular	-	-	-	89.5	-	-	-	16[a]

*Values with different alphabets in superscripts along the same column are statistically different ($p \leq 0.05$).

Table 3. Distribution of colony elevation types among *Candida* species.

Colony Elev.	Percentage Distribution							
	C.glab.	*C. trop.*	*C. guill.*	*C. alb.*	*C. pseu.*	*C. para.*	*C.alb. var stell.*	%Total*
Raised	100	100	100	70.5	100	100	100	95.1[a]
Flat	-	-	-	29.5	-	-	-	5.0[b]

*Values with different alphabets in superscripts along the same column are statistically different ($p \leq 0.05$).

showed a higher frequency of isolation of non-*albicans* contrary to several reports (Enweani *et al.,* 1987; Orazio and Criseo, 2009; Babic and Hukic, 2010) that *C. albicans* is the most frequently isolated species, an indication of a possible change in the distribution and ecology of *Candida* species. So there is need for regular updates on the biology of *Candida*.

The variations observed in colony morphology (especially with regards to species specificity) in *C. pseudotropicalis*, *C. guilliermondii*, *C. parapsilosis* and *C. stellatoidea* (colony surface), and intra species variations (colony surface in *C. glabrata*, *C. albicans* and *C. tropicalis*, and colony consistency in *C. tropicalis*) give an insight into the great diversity that exists among *Candida* species. The difference between the DS and DP in *C. tropicalis* and

Table 4. Distribution of colony colors among *Candida* species.

Colour	Percentage of *Candida* species							
	C.glab.	*C. trop.*	*C. guill.*	*C. alb.*	*C. pseu.*	*C. para.*	*C.alb. var stell.*	%Total*
White	-	-	-	-	-	3.0	-	3.0 [a]
Off-white	30.0	22.9	22.0	17.6	17.6	-	-	93.0 [b]
Brownish	-	-	-	-	-	-	0.8	3.5 [a]

*****Values with different alphabets in superscripts along the same column are statistically different ($p \leq 0.05$).

between the W and DS in *C. albicans* was significant ($p \leq 0.05$). The total percentage of the DS was also significantly higher than the other types. The creamy consistency was significantly higher in distribution, which means that most *Candida* colonies are creamy. All the colonies showed raised colonies except *C. albicans* which had some flat colonies as well; this intra-specific variation in *C. albicans* may represent a morphological shift in its host colonization strategy. Although most of the isolates were off-white in colour, the small percentage showing white and brownish colours is important in the preliminary identification of species based on colony morphology.

This is the first all-encompassing documented report on colony morphology variations in *Candida* species and has added more information to the available data on *Candida* morphology and diversity, an essential tool in the understanding and control of these species.

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REFERENCES

Ahmad, S., Khan, Z., Mokaddas, E. and Khan, ZU. 2004. Isolation and molecular identification of *Candida dubliniensis* from non-human immuno-deficiency virus-infected patients in Kuwait. Journal of Medical Microbiology. 53:633-637.

Alexopoulos, CJ. and Mims, CW. 1979. Introductory Mycology. (3rd ed.) John Wiley and Sons, New York. pp632.

Babic, M. and Hukic, M. 2010. *Candida albicans* and non-*albicans* species as etiological agent of vaginitis in pregnant and non-pregnant women. Bosnian Journal of Basic Medical Sciences. 10 (1):89-97.

Carlsen, G. 2001. The *Candida* Yeast Answer. *Candida* Wellness Center, Provo, Utah. pp50.

Dede, APO. and Okungbowa FI. 2009. Effect of pH on *in vitro* yeast-mycelial dimorphism in genitourinary *Candida* spp. Bioscience Research Communications. 21(4):177-181.

Enweani, IB., Ogbonna, CI. and Kozak, W. 1987. The incidence of candidiasis amongst the asymptomatic female students of the University of Jos, Nigeria. Mycopathologia. 99 (3):135-141.

Houang, ET., Chu, KC., Koehler, A. and Chen, ATF. 1997. Use of CHROM-Agar *Candida* for genital specimens in the Diagnostic laboratory. Journal of Clinical Pathology. 50 (7):563-565.

Leboffe, MJ. and Pierce, BE. 1996. A Photographic Atlas for the Microbiology Laboratory (1st ed.). Morton Publishing Company, Colorado, USA. pp129.

Lipperheide, V., Bikandi, J., Garcia-Fernandez, JF., Quindos, G. and Ponton, J. 2002. Colony variation in *Candida glabrata* isolates from patients with Vaginitis. Revista Iberoamericana de Micologia. 19:161-164.

Okungbowa, FI., Isikhuemhen, OS. and Dede, APO. 2003. The distribution frequency of *Candida* species in the genitourinary Tract among symptomatic individuals in Nigerian cities. Revista Iberoamericana de Micologia. 20:60-63.

Okungbowa, FI., Dede, APO. and Isikhuemhen, OS. 2009. Cell Morphology variations and budding patterns in *Candida* isolates. Advances in Natural and Applied Sciences. 3(2):192-195.

Orazio, R. and Criseo, G. 2009. Molecular epidemiology of *Candida albicans* and its closely related yeasts *Candida dubliniensis* and *Candida africana*. Journal of Clinical Microbiology. 47(1):212-214.

Rex, JH., Pfaller, MA., Barr, AL., Nelson, PW. and Webb, CD. 1995. Antifungal Susceptibilities Testing of isolates from a Randomized Multicenter Trial of Fluconazole versus Amphotericin B as Treatment of Non-neutropenic patients with candidemia. Antimicrobial Agents and Chemotherapy. 39 (1): 40-44.

Rodgers, CA. and Berdall, AJ. 1999. Recurrent vulvovaginal candidiasis. Why does it occur? International Journal of STD and AIDS. 10 (7):435-439.

Taylor, DJ.,Green, NPO. and Stout, GW. 1998. Biological Science (3rd ed.). Cambridge University Press, Cambridge. pp984.

Vidotto, V., Mantoan, B., Pugiliese, A., Ponton, J., Quidos, G. *et al*. 2003. Adherence of *Candida albicans* and *C. dubliniensis* to buccal cavity and enzymatic activity (proteinase and phospholipase) of *Candida albicans*. Pesq. Odont. Bras. 14 (2):119-122.

Yang, Y., Cheng, H., Lo, H. and Tsary, T. 2006. Distribution and antifungal susceptibility of *Candida* species isolated from different age populations in Taiwan. Medical Mycology. 44:237-242.

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