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Genotypes of Candida albicans from Stool Samples of HIV Sero Positive and HIV Negative Individuals in Nairobi, Kenya

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Abstract
Candida albicans is one of the commonest opportunistic yeast pathogens that infect both healthy and immune-compromised individuals. Currently there are five recognized genotypes of *C. albicans* based on the 25 rDNA gene. Different genotypes of *C. albicans* have been shown to differ in their virulence and susceptibility to antifungal drugs and, thus accurate identification of the infecting genotype is essential for guiding antifungal therapy. The aim of the study was to determine the genotypes of *C. albicans* from stool samples of HIV sero positive and HIV negative individuals and ascertain whether there is an association between HIV status and genotype distribution. A total of forty four (44) *Candida albicans* isolates from the stool samples of 28 HIV sero positive patients attending an outpatient HIV/AIDS management clinic and 16 HIV negative individuals attending a hospitality industry support program at Kenya Medical Research Institute (KEMRI) were genotyped using PCR primer pairs that span the transposable intron region of the 25S rDNA. Genotype A *C. albicans* was the most prevalent type (61.4%) followed by genotype C (11.4%) and genotype B (4.5%). Genotype B *C. albicans* was not isolated from HIV negative individuals. There was no significant difference in the genotype distribution of *C. albicans* isolates from HIV sero positive and HIV negative individuals ($p=0.304$). Ten (22.7%) *C. albicans* isolates could not be genotyped using the transposable region primers an indication of possible new genotypes. Genotype A is the most predominant *C. albicans* isolate both in HIV sero positive and HIV negative individuals and there is no predilation of any genotype to HIV infected. There is a possibility of new genotypes of *C. albicans* and sequence analysis may be warranted.
Keywords: Genotypes, *C. albicans*, stool, HIV, Kenya.

1. Introduction

Candida albicans is a normal flora of the mucocutaneous cavities of the human skin, vagina and intestines (Adams *et al.*, 2010). However under altered physiological and pathological conditions like pregnancy, infancy, diabetes, cancer, prolonged broad-spectrum antibiotic use, steroidal chemotherapy and AIDS it can take advantage of the weakened immunity to cause infections (Van Burik & Magee, 2001). Fungal infections particularly those due to yeast have increased remarkably in the past few decades (Bii *et al.*, 2002). Majority of these yeast-related mycoses are associated with members of the genus *Candida* and *Cryptococcus* (Pfaller & Diekema, 2007). Within the genus *Candida*, majority of infections are caused by *C. albicans* (Papas, 2006) although non-*albicans Candida* species are increasingly becoming significant (Bii *et al.*, 2002). This has necessitated identification of yeasts to species and genotype level.

Genotyping of *C. albicans* strains is epidemiologically important as it enables recognition of infection outbreaks, detection of cross-transmission, determination of the infection source, recognition of virulent strains and emerging drug resistance strains (Garcia-Hermoso *et al.,* 2007). Over time, several typing techniques have been developed to genotype *C. albicans* strains such as, random amplified polymorphic DNA (RAPD), restriction length polymorphic DNA, electrophoretic karyotyping, microsatellite length polymorphism (MLP), multilocus sequence typing (MLST) (Garcia-Hermoso *et al.,* 2007), Polymerase chain reaction Melting Profile (PCR-MP) (Krawczyk *et al.*, 2009) and high-resolution DNA melting (HRM) analysis (Costa *et al.,* 2010). Although these techniques demonstrate a high discriminatory potential at the strain level, they consume a lot of time and are very expensive.

In the present study, a PCR-based technique that utilizes specific primer pairs that span the regions of the transposable group I intron of 25S rDNA gene were used determine the genotypes of forty four (44) *C. albicans* strains from stool samples of HIV sero positive and HIV negative individuals and to ascertain whether there is an association between HIV status and genotype distribution.

2. METHODS

Sources of *C. albicans* isolates

A total of forty four (44) *C. albicans* isolates were used. The isolates were obtained from stool samples of consented HIV sero positive patients attending Family Aids Care and Educational Services (FACES) clinic at the Center for Respiratory Disease Research (CRDR), Kenya Medical Research Institute (KEMRI) and HIV negative individuals attending Hospitality Industry Support Program (HISP) at Center for Microbiology Research (CMR), KEMRI between November 2013 and March 2014. The clinical isolates were identified as *C.*

albicans based on their morphological characteristics such as germ tube formation at 35°C on human serum, growth at 45°C on Sabouraud Dextrose Agar (SDA) (Oxiod, UK), colony color on CHROMagar *Candida* media and Chlamydospores production on Corn Meal Agar (CMA). The isolates were confirmed using Analytical profile index (API 20C AUX) strips (Biomeriux, Etiole, France).

Genotyping of *Candida albicans* isolates

Candida albicans isolates were genotyped as described by McCullough *et al*, 1997. The DNA isolation was done using Phenol/chloroform extraction procedure. Briefly, a colony of each *C. albicans* isolate was suspended in 100 µl lysis buffer in an eppendorf tube, vortexed for 10 s and then boiled at 100°C for 20 min to lyse the yeast cells and extract DNA. One hundred microlitres (100µl) of 2.5 M Sodium acetate was added and the mixture was kept at -20^{O}C for 1 h. After centrifugation at 12000 rpm for 5 minutes at 4°C, the supernatant was transferred to a sterile tube and an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added, vortexed and centrifuged for 5 minutes. The supernatant was transferred to a new sterile tube and an equal volume of chloroform/ isoamyl alcohol (24:1) was added and vortexed. After centrifugation for 5 minutes the supernatant was transferred to a sterile tube and an equal volume of isopropanol was added and vortexed and centrifuged for 15 min. The supernatant was discarded and 150µl of 70% cold ethanol was added to the precipitate, mixed gently and centrifuged for 2 min. The supernatant was discarded and the pellet washed with 70% ethanol, air dried and then re-suspended in 100 *µl* TE.

PCR primers

The PCR primer pairs that span the transposable intron region of the 25S rDNA were used. They were: CA-INT-L (5'ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3') and CA-INT-R (5'CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3').

PCR Amplification

In the PCR reaction, 1 *µl* re-suspended DNA template was used for each sample analyzed along with 0.5 *µl* Taq polymerase, 1 *µl* of each of the CA-INT'L/ CA-INT'R primers, 1.5 mM $MgCL_2$, 200 *µ*m dATP, 200*µ*m dTTP, 200 *µ*m dGTP, and 200 *µ*m dCTP. The reaction volume was topped up to 50 *µl* with sterile distilled water. The reaction was performed in an automated thermal cycler (PERKIN ELMER) under the following conditions: Denaturation at 94°C for 3 min then 30 cycles of 1 min at 94^{O}C, 1 min at 65^{O}C and 2.5 min at 72^{O}C min and final extension at 72^{O}C for 10 minutes.

Electrophoresis

Ten microlitres (10 *µl)* of the amplified products were electrophoresed on horizontal 1.5% agarose gel in 1 X TAE (40 mM Trisacetate, 0.2 Mm EDTA) for 30 min at 100 V. Thereafter the gel was stained with ethidium bromide for 5 min, and then the bands were visualized by ultraviolet (UV) trans-illumination (JENCONS-PLS, Japan) at 302 nm. *Candida albicans* ATCC 90028 genotype A and distilled water (instead of DNA template) were included in each PCR reaction as the positive and negative control respectively.

3. Results

Genotypic analysis of the 44 *Candida albicans* isolates obtained from stool samples of 28 HIV sero positive and 16 HIV negative individuals indicated that genotype A was the most prevalent type (61.4%) followed by genotype C (11.4%) and genotype A (4.5%). Genotypes D or E *C. albicans* were not detected in this study. The genotypes of 10 (22.7%) *C. albicans* isolates could not be determined as they did not show any specific band. Genotype B was not isolated from the stool samples of HIV negative individuals. There was no significant difference in the genotype distribution of *C. albicans* isolated from the stool samples of HIV sero positive and HIV negative individuals (*p=0.304*) (Table 1, Figure 1).

4. Discussion

Candida albicans is the commonest opportunistic fungal pathogen in humans (Bai, 2014) and is responsible for over 80% of all *Candida* infections (Bii *et al.*, 2009). Advances in molecular biology have enhanced our understanding of the epidemiology, virulence and genetic diversity of *Candida* and has promoted the development of logical disease control measures (McCullough *et al.*, 1999; Zhu *et al.,* 2011).The use of 25S rDNA group 1 intron to genotype *C. albicans* distinguishes 5 genotypes including genotypes A, B, C, D (*C. dubliniensis*) and E (McCullough *et al.*, 1997). This genotyping technique is simple, accurate and can effectively be used to analyze many isolates (Mane *et al.*, 2012).

In the present study we determined the genotypes of 44 *C. albicans* isolates recovered from the stool samples of HIV sero positive and HIV negative individuals using PCR primers that span the intron I group of the 25S rDNA gene. Based on the length of the amplified PCR product the isolates were classified into three

genotypes: Genotype A, exhibited a 450-bp product; genotype B, exhibited an 840-bp product and genotype C, exhibited 450-bp and 840 bp products (McCullough *et al.*, 1999). No atypical profile genotypes were recovered including genotypes BC and D previously reported (Bii, *et al.*, 2009).

Overall genotype A was identified as the most prevalent type (61.4%) followed by genotype C (11.4%) and genotype B (4.5%). These finding is consistent with findings by Bii *et al.* (2009), who reported that genotype A *C. albicans* was the most predominant type obtained from clinical samples of hospitalized patients followed by genotype C and genotype B. Mane *et al*. (2012), reported in India that genotype A was the most predominant type from oral swabs of 335 individuals (HIV positive and HIV negative) attending a National AIDS Research Clinic in India. The distribution of *C. albicans* into the 3 genotypes was similar to those previously reported in China (Zhu *et al.*, 2011), India (Mane *et al.*, 2012) and Turkey (Karahan *et al.*, 2004).

Irrespective of HIV status *C. albicans* genotype A was the most prevalent type. In HIV sero positive individuals, Genotype A had a higher prevalence of 53.6%. The predominance of genotype A *C. albicans* among HIV sero positive individuals has been reported previously (Kumar *et al.*, 2009; Mane *et. al*, 2012). In HIV negative individuals' genotype A accounted for 75% of all *C. albicans* strains. This is comparable to the findings by Mane *et al.* (2012), who reported a genotype A *C. albicans* prevalence of 71.4% in isolates obtained from HIV negative individuals. Genotype C was second most prevalent type isolated from both HIV sero positive and HIV negative individuals. This is consistent with findings by Mane *et al.* (2012), and Kumar *et al*. (2009). Genotype B, the least prevalent type was not recovered from HIV negative individuals and these coincides with findings by Mane *et al*, (2012).There was no significant difference ($p=0.304$) in the genotype distribution of *C. albicans* isolates from the stool samples of HIV sero positive and HIV negative individuals. This could mean that the genotypes of *C. albicans* found in HIV sero positive are similar to those found in HIV negative individuals.

Although all genotyping conditions outlined by McCullough *et al.* (1997), were strictly adhered to, the genotypes of 22.7% API 20 C aux confirmed *C. albicans* isolates could not be determined as they did not produce any specific band. Similar results by Bii *et al.* (2009), also noted that 12% *C. albicans* isolates did not yielded any specific band therefore could not be typed. Failure by some strains of *C. albicans* to yield a band would suggest the existence of other genotypes of *C. albicans* in Kenya besides those described previously (McCullough *et al.*, (1997).

Genotype D (*C. dubliniensis*) is frequently isolated from immune-compromised individuals especially HIV sero positive patients (Sullivan *et al.*, 2004). Genotype E is very rare and only one isolate has been reported previously (Liu *et al.*, 2009). Failure of the study to isolate Genotype D or genotype E *C. albicans* could be due to the small sample size used. However further research involving many clinical samples would give more insights into the genotypic diversity and epidemiology of *C. albicans* in Kenya. In addition, sequence analysis might shade more light on the genotype diversity of *C. albicans* especially those which could not be typed.

5. Conclusion

The genotypes of *C. albicans* isolated from HIV sero positive individuals are similar to those isolated from HIV negative individuals. There is need for further research involving a larger sample size and use of sequence analysis to clarify the presence of atypical or new genotypes of *C. albicans* in Kenya.

References

Adams, A., Kumar, S., Clauson, M. & Sahi, S. (2011), "Anti-yeast activities of *Origanum* oil against human pathogenic yeasts", *Advances in Bioscience and Biotechnology,* 2:103-107.

Bai, F.Y. (2014), "Association of genotypes with infection types and antifungal susceptibilities in *Candida albicans* as revealed by recent molecular typing strategies", *Mycology*, 5(1):1–9.

Bii, C.C., Kangogo, M.C., Revathi, G. & Wanyoike M.W. (2009), "Genotypes of *Candida albicans* from clinical sources in Nairobi Kenya", *African Journal of Microbiology Research,* 3(9): 475-477.

Bii, C.C., Ouko, T.T., Amukoye, E. & Githinji, L.W. (2002), "Antifungal drug susceptibility of *Candida albicans", East Africa Medical Journal,* 79(3): 143-5.

Costa, J.M. Garcia-Hermoso, D., Olivi, M., Cabaret, O., Farrugia, C., Lecellier, G., Dromer, F. & Bretagne, S. (2010), "Genotyping of Candida albicans using length fragment and high-resolution melting analyses together with minisequencing of a polymorphic microsatellite locus", *Journal of Microbiological Methods*, 80(3):306-309.

Garcia-Hermoso, D., Cabaret, O., Lecellier, G., Densnos-Ollivier, M., Hoinard, D., Raoux, D., Costa, J.M., Dromer, F. & Bretagne, S. (2007), "Comparison of Microsatellite Length Polymorphism and Multilocus Sequence Typing for DNA-Based Typing of *Candida albicans", Journal of clinical Microbiology,* 45(12):3958-3963.

Karahan, Z. C., Guriz, H., Agirbash, H., Balaban, N., Göçmen, J. S., Aysey, D. & Akar, N. (2004), "Genotype distribution of *Candida albicans* isolates by 25S intron analysis with regard to invasiveness", *Mycoses*,

479(11-12): 465–469.

Kumar, C. P. G., Menon, T., Rajasekaran, S., Sekar, P. & Prabu, D. (2009), "Carriage of Candida species in oral cavities of HIV infected patients in South India", *Mycoses,* 52(1):44-48.

Krawczyk, B., Leibner-Ciszak, J., Mielech, A., Nowak, M. & Kur, J. (2009), "PCR melting profile (PCR MP)--a new tool for differentiation of Candida albicans strains", *BMC infectious disease*, 9:177-184.

Liu, Y., Kang, Y., Yokoyama, K., Gonoi, T. & Mikami, Y. (2009), "Molecular differentiation and antifungal susceptibility of Candida albicans isolated from patients with respiratory infections in Guiyang Medical College Hospital, China", *Nihon Ishinkin Gakkai Zasshi*, 50(3):175-8.

Mane, A., Gaikwad, S., Bembalkar, S. & Risbud, A. (2012), "Increased expression of virulence attributes in oral *Candida albicans* isolates from human immunodeficiency virus-positive individuals", *Journal of Medical Microbiology,* 61(2): 285-290.

McCullough, M.J., Clemons, K.V. & Stevens, D.A. (1997), "Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea", Journal of Clinical Microbiology,* 37:417–421.

McCullough, M.J., Clemons, K.V. & Stevens, D.A. (1999), "Molecular and phenotypic characterization of genotypic *Candida albicans* subgroups and comparison with *Candida dubliniensis* and *Candida stellatoidea", Journal of Clinical Microbiology,* 37(2):417–421.

Papas, P. G. (2006), "Invasive Candidiasis", *Infectious Disease,* 3:485-506.

Pfaller, M. A. & Diekema, D. J. (2007), "Epidemiology of Invasive Candidiasis: a persistent public health problem", *Clinical Microbiology Review,* 20(1): 133-163.

Sullivan, D.J., Moran, G.P., Pinjon, E., Al-Mosaid, A., Stokes, C., Vaughan, C. & Coleman, D.C. (2004), "Comparison of the epidemiology, drug resistance mechanisms, and virulence of Candida dubliniensis and Candida albicans", *FEMS Yeast Research*, 4(4–5):369–76.

Van Burik, J.A. & Magee, P.T. (2001), "Aspects of Fungal Pathogenesis in Humans", *Annual Review of Microbiology,* 55:743-772

Zhu, X., Dong, N., Wang, Q., Cheng, B., Zhu, L., Tao, J., Zhu, G. & Bo, P. (2011), "Genotyping and antifungal susceptibility of Candida albicans clinical isolates from Yangzhou region in China", *African journal of Microbiology Research*, 5(25): 4406-4410.

Table 1: Genotypes of 44 *C. albicans* isolated from stool samples of HIV sero positive and HIV negative individuals.

	HIV status			
Genotypes	**HIV sero positive n (%)**	**HIV negative n (%)**	**Total n (%)**	
Genotype A	15(53.6)	12(75)	27(61.4)	**P-value= 0.304**
Genotype B	2(7.1)	0(0)	2(4.5)	
Genotype C	4(14.3)	1(6.3)	5(11.4)	
*	7(25)	3(18.8)	10(22.7)	
Total	28(100)	16(100)	44(100)	

(N/B* Confirmed *C. albicans* isolates that yielded no band, n: No of isolates; (%): Percentage)

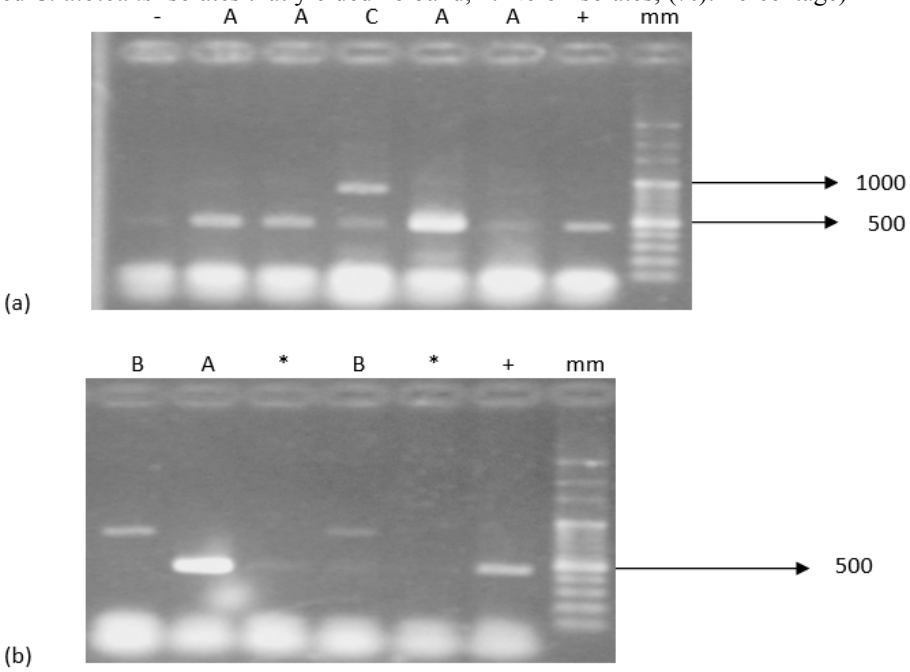

Figure 1: Representative gels showing *Candida albicans* genotypes. A, B, C represent different genotypes, * No specific band amplified, + *C. albicans* ATCC 90028 genotype A, - negative control, mm-wide range DNA ladder.