Short Communication

Genotypes of *Candida albicans* from clinical sources in Nairobi Kenya

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Accepted 4 August, 2013

The present study genotyped *Candida albicans* isolates from clinical sources in Nairobi. The isolates were from blood, sputum, swabs, urine and catheters tips isolated between 2000 - 2005. Genotypic analysis was done using primer pairs that span the site of the transposable intron in the 25S rDNA. Genotypic analysis indicated that 60% of the *C. albicans* were genotype A exhibiting one band size of 450 base pair. This was followed by genotype C (16%) with two bands of approximately 450 and 650 amplicon sizes. Eight percent were genotype B with one band size of 650 base pairs. Four percent of the isolates gave a faint band of approximately 550 base pairs. These isolates did not correspond with any of the earlier described genotypes; A, B, C, D and E therefore we placed it in a new genotype BC.

Our local *C. albicans* isolates strain showed slightly different amplicon sizes as those reported previously. Genotype E which corresponds to *Candida dubliniensis* with amplicon size of 1080 bp was not detected and confirms no reports of *C. dubliniensis* in Kenya. Although 12% of the isolates were confirmed as *C. albicans*, no specific band was amplified and could not be genotyped. More extensive work to determine the presence of *C. dubliniensis* and other possible genotypes of *C. albicans* may be necessary.

Key words: *Candida albicans*, genotypes, clinical, Kenya.

INTRODUCTION

*Candida albicans* is one of the most frequently isolated yeasts in clinical laboratories. Different studies have shown that this organism accounts for up to 80% of the yeasts recovered from clinical specimens (Rex et al., 2000). *C. albicans* has been associated with infections, as well as colonization, in both immunocompromised and immunocompetent patients (Wade, 1993). Recent advances in molecular biology - based technology enable detailed analysis of the genetic diversity of *C. albicans*, and some groups of *C. albicans* strains have been genetically characterized and reported (Kirkpatrick et al., 1998; Kurtzman and Robnett, 1998; Mercure et al., 1993). McCullough et al., (1997) reported that a PCR primer designed to span the 25S rRNA gene (rDNA) region can classify *C. albicans* strains into four genotypes on the basis of the amplified PCR product length: genotype A (450 bp product), genotype B (840 bp product), genotype C (450 and 840 bp products), and genotype D (1,080 bp product). In their report, they confirmed that *C. albicans* genotype D belongs to the same taxon as *Candida dubliniensis*. We applied the same primers and conditions to genotype *C. albicans* from the clinical sources in Kenya because of cost effectiveness and our lack of more discriminatory alternatives.

The available literature shows that no single phenotypic test is highly effective in identifying *Candida* spp. A Combination of tests is therefore necessary for identification and molecular techniques are faster and specific in identification of *Candida* spp (Pfaller, 1995).

The aim of the study was to determine *C. albicans* genotypes using primers that span the site of transposable intron in the 25 rDNA. This is the preliminary report.
on the genotypic distribution of *C. albicans* from clinical setting in Kenya and an attempt to elucidate the presence of *C. dubliniensis* in Kenya.

**MATERIALS AND METHODS**

**Sources of isolates**

This study investigated a total of 150 *Candida* isolates from Health Institutions in Nairobi, Kenya. They were from culture collections from previous studies in Mycology Laboratory, Kenya Medical Research Institute. The isolates were recovered from swabs, urine, sputum, aspirates, Cerebral Spinal Fluid (CSF), blood and bile of both HIV positive and HIV negative adult male and female hospitalized patients. Majority of the isolates were from high vaginal swabs (HVS) of female patients with vaginal candidiasis.

**Confirmation of isolates**

Preliminary identification of *C. albicans* were done using ChromAgar and Germ tube test. Analytical profile index (API 20 C AUX) (Bio Merieux, Etoile, France) was used for confirmatory purposes. The API strip was inoculated with the test yeast and incubated at 37°C for 24 - 48 h. After incubation, numerical profiles were constructed from the reaction patterns and were used to obtain identification results with the analytical profile index.

**Genotype analysis**

*C. albicans* isolates were genotyped using primers and conditions described by McCullough et al., 1997. DNA extraction was done using Phenol/Chloroform method. Briefly a small colony of each *C. albicans* isolate was picked and suspended in 100 µl of lyses buffer in appendeford tube. This was vortexed for 10 s and boiled at 100°C for 1 min. 100 µl of 2.5 M Sodium acetate was then added and kept at -20°C for 1 h. This was centrifuged at 13000 rpm for 5 min at 4°C using a refrigerated centrifuge (Tomy Seiko CO., LTD Japan). The supernatant was transferred into a new tube and an equal volume of phenol / chloroform / isoamyl alcohol was added, vortexed and centrifuged at 12000 rpm for 5 min at 4°C. The supernatant was transferred into a new tube and an equal volume of Chloroform / Isoamyl alcohol (24:1) added. The mixture was vortexed and centrifuged at 12000 rpm for 5 min at 4°C. The supernatant was then transferred to new tube and isopropanol added vortexed and centrifuged at 12000 rpm for 15 min at 4°C. The supernatant was discarded and 150 µl of 70% cold ethanol added, mixed gently and centrifuged at 12000 rpm for 2 min at 4°C. The supernatant was discarded and the pellet was vacuum dried for 9 min after which the pellet was dissolved in 100 µl TE.

**Polymerase Chain reaction (PCR)**

Genotypic analysis was done using primer pairs that span the site of the transposable intron in the 25S rDNA. Primers and sequence used were; CA-INT-L (5’-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3’) and CA-INT-R (5’-CCT TGG CTT GGG TTT CGC TAG ATA GTA GAT-3’).

PCR amplification was done in a 50 µl volume containing 1 µl of each primer, 1.5 Mm MgCl2, 0.5 µl of Taq polymerase, 200 µm dATP, 200 µm dCTP, 200 µm dGTP and 200 µm dTTP and 1 µl of template topped up to 50 µl with sterile distilled water. The mixture was subjected to thermocycling using an automated thermal cycler (PERKIN ELMER). The following PCR conditions were used: denaturation for 3 min at 94°C before 30 cycles of 94°C for 1 min, 65°C for 1 min and 72°C for 2.5 min final extension at 72°C for 10 min. The PCR products were then electrophoresed through 1.5% agarose gel in 1x TAE buffer (40 mM Tris-acetate, 0.2 Mm EDTA) for 40 min at 100 V. Bands were visualized by UltraViolet transilluminator (JENCONS-PLS, Japan) after Ethidium bromide staining. The samples were tested in triplicate. *C. albicans* ATCC 90028 genotype A was used as positive control and water instead of template to serve as negative control.

**RESULTS**

Genotypic analysis of *C. albicans* isolates indicated that 60% belonged to genotype A (Figure 1b). In this genotype only one band of 450 base pair was amplified. The band size was similar to that of reference strain *C. albicans* ATCC 90028 genotype A which was used as positive control. The second common genotype was C (16%) which exhibited two bands of approximately 450 and 650 base pairs (Figure 1a). Eight percent were categorized as genotype B with one band of approximately 650 base pairs. Four percent of the isolates gave a faint band of approximately 550 base pairs (Figure 1 c). These isolates did not correspond to any of the earlier described genotypes A, B, C, D and E; we therefore placed it in a new genotype BC. However, 12% of API confirmed *C. albicans* did not show any specific band and could not be genotyped.

**DISCUSSION**

In the recent years the number of serious opportunistic yeast infections, particularly in immunocompromised patients has increased dramatically (Richardson and Warnock, 2003). Among them is *Candida albicans*, which accounts for over 80% of yeast infections. We genotyped *C. albicans* from clinical sources in Nairobi and identified four genotypes namely; A, B, C, and BC. This is the first study documenting the genotype distribution of *C. albicans* in Kenya. Although all conditions used were same as that described by McCullough et al., 1997, we noted with interest the slight differences in the amplicon sizes with that previously reported (Tamura et al., 2001). As expected the *C. albicans* genotype A amplified one band of 450 base pairs. However genotype B which was supposed to give one band of 840 base pair band amplified one band of 650 base pairs instead. In genotype C, two bands of 450 and 650 instead of 450 and 840 base pairs were amplified. There were 4% of the *C. albicans* isolates in which a faint band of 550 base pair was amplified which we could not categorize as either genotype D or E, with expected amplicon size of 1,080 and 1400 base pairs respectively. These strains were designated *C. albicans* genotype BC. Genotype E with amplicon size of 1,080 base pair which represent *C. dubliniensis* was not detected which con-
firms our phenotypic characterization that did not identify any C. dubliniensis (Sullivan and Coleman, 1998).

Genotypic analysis not only categorizes C. albicans into five genotypes but can also discriminate against C. dubliniensis. According to Sullivan et al., (1998), C. dubliniensis is a significant opportunistic pathogen worldwide that is phylogenetically closely related to C. albicans (Kirkpatrick et al., 1998). Evidence of inducible stable fluconazole resistance in vitro in C. dubliniensis strain has been demonstrated in immunocompromised patients receiving long-term fluconazole prophylaxis (Mercure and Montplaisir, 1993; Moran et al., 2003). This situation has necessitated that clinical laboratories be able to genotype and discriminate between C. albicans and C. dubliniensis as this has clinical implications.

Although all the conditions were carried out as described by McCullough (1997), it was noted that some (12%) of confirmed C. albicans isolates yielded no specific band and therefore could not be genotyped using the primer set used. These could be an indication that there could be other genotypes of C. albicans circulating in Kenya different from that reported elsewhere. However, other confirmatory molecular techniques such as sequence analysis are needed. The difference in amplicon sizes with that reported in Japan could be that primers used were initially designed using C. albicans strains from elsewhere and did not include strains from Africa or Kenya. Strains from different geographical regions have been known to exhibit slight differences in genotypic features.

Genotype A was the most predominant (60%) consistent with other studies in Japan showing that majority of C. albicans from clinical sources belongs to genotypes A followed by genotypes B, C and D respectively. However, in the present study the prevalence distribution was: genotype A (60%), C (16%), B (8%) and BC (4%). As these findings are preliminary, there is need to genotype more isolates using more discriminatory techniques and more studies to elucidate the presence of C. dubliniensis in Kenya.

REFERENCES


Figure 1. Representative gel showing different genotypes of Candida albicans. A, B, C, BC represent different genotypes.* No specific band amplified, + Candida albicans ATCC 90028 genotype A and -negative control. MM-wide range DNA ladder.