

Characterization of Candida isolates from South African pregnant and non-pregnant women

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BACKGROUND

Candida species are opportunistic organisms that cause several infections in women. Studies have found that vaginal Candida infections affect up to 75% of women in their lifetime. Symptoms associated with Candida infection include: redness around the genital area, inflammation of the genital tract, itchiness and thick white discharge

Despite treatment of vaginal Candida infections, women still experience a high reoccurrence of Candida infections. This reoccurrence could be the result of excessive use of antifungal drugs and overuse of broad-spectrum antibiotics which leads to antimicrobial resistance.

Different studies have shown candida resistance to amphotericin B. Despite, *C.albicans* is responsible for most vaginal Candida infections (70-90%), there has been an increase in other Candida isolates displaying antimicrobial resistance patterns. Characterization of Candida isolates from women who are culture positive is very important for identifying the species of Candida responsible for the infection as well as determining antimicrobial susceptibility profiles and resistance mechanisms.

The ABC genotyping method is mostly used for the characterization of *C. albicans* whereby different band sizes are used to determine the genotype of isolates. The isolates are classified into genotypes: A (450bp), B (840bp), C (450bp and 840bp), and D (1080bp) after amplification of the 25S ribosomal DNA by the Polymerase Chain Reaction (PCR).

In this study, the antimicrobial resistance profiles of Candida clinical isolates to amphotericin B were investigated. The ABC genotyping method was performed on the isolates and the correlation between genotypes and clinical factors and genotypes and resistance profiles were also determined.

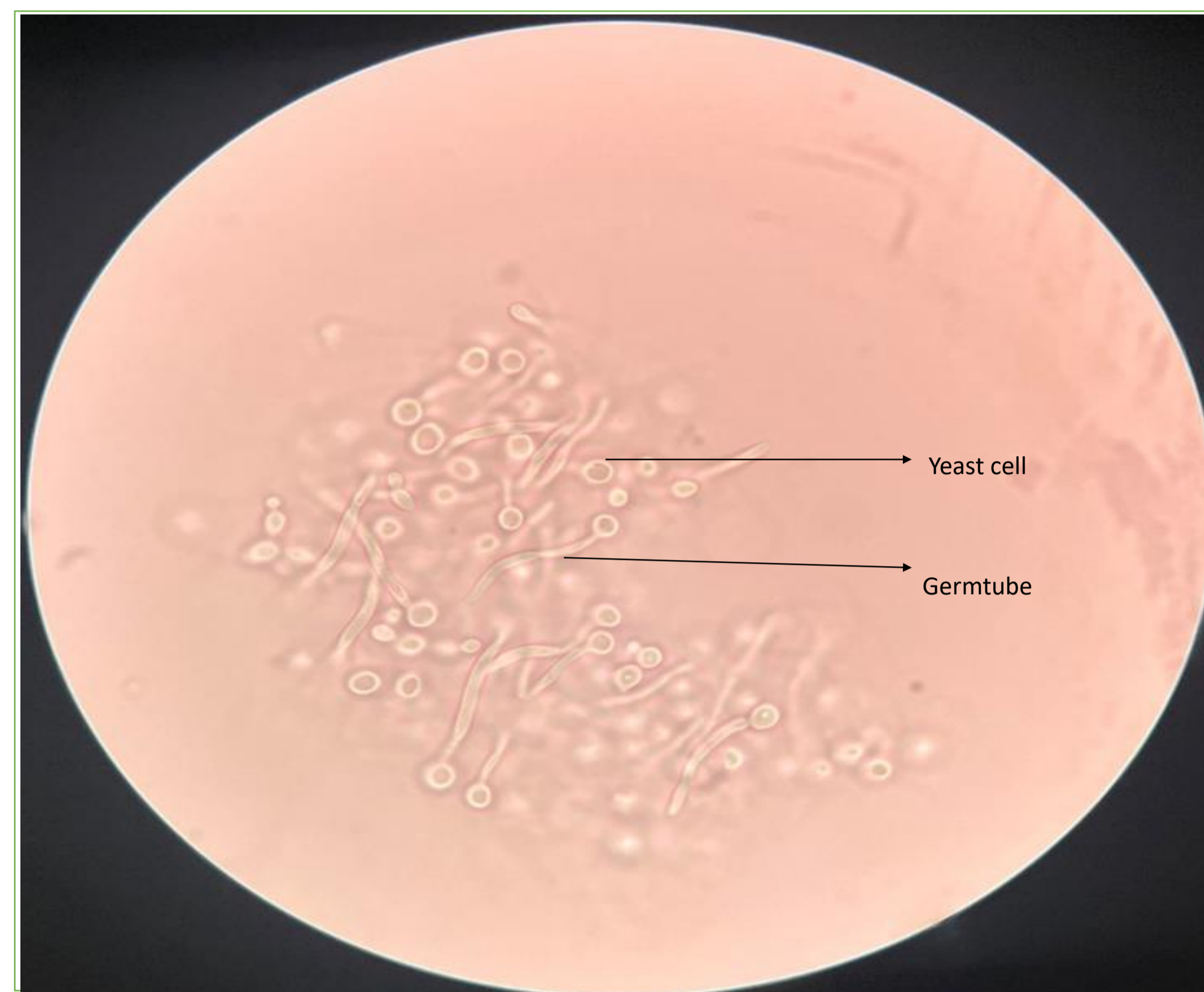


Figure 1: Microscope slide depicting the results of the germ tube test which was conducted. The slide was viewed with oil immersion under 100X magnification

METHODS

STUDY POPULATION	<ul style="list-style-type: none"> Biomedical Research Ethics Committee (BREC), UKZN 31 pregnant and 41 non-pregnant women from King Edward VIII Hospital Provided written informed consent and vaginal swab
SAMPLE PROCESSING AND DNA EXTRACTION	<ul style="list-style-type: none"> Vaginal swabs resuspended in 2 ml of phosphate buffered saline (PBS) Suspension centrifuged at 14,000 rpm for 10 mins Pellets subjected to DNA extraction using the PureLink Microbiome Kit
CONFIRMATION ASSAY OF C. ALBICAN	<ul style="list-style-type: none"> Detected using the Applied Biosystems™ TaqMan® Assay Primers and probes specific for <i>C. albicans</i> (CA-INT-L (5'-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3') and CA-INT-R (5'-CCTTGG CTG TGG TTT CGC TAG ATA GTA GAT-3')) Performed on the QuantStudio™ 5 Real-Time PCR detection system
THE GERM TUBE TEST	<ul style="list-style-type: none"> A single colony of the culture growing on the SDA plate was added to the serum and mixed. The tubes were incubated at 37°C for 2 to 4 hours. After incubation, a drop of the sample was placed on a glass slide and viewed under the microscope. A positive germ tube result was observed when there were short hyphal. A negative result was observed when there were no hyphal
GENOTYPING	<ul style="list-style-type: none"> The isolates were typed using the ABC genotyping method. Using the already extracted DNA from the isolates, the 25S rDNA gene was amplified using the following primers: CA-INT-L (5'-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3') and CA-INT-R (5'-CCTTGG CTG TGG TTT CGC TAG ATA GTA GAT-3')
ANTIFUNGAL TESTING	<ul style="list-style-type: none"> Susceptibility testing was performed using the broth microdilution assay to measure the minimal inhibitory concentrations (MICs) for <i>C. albicans</i> clinical isolates to amphotericin B Briefly, two-fold serial dilutions of amphotericin B were performed in Mueller Hinton broth. The resulting concentrations ranged from 0.5 to 32µg/ml. The <i>C. albicans</i> ATCC 10231 strain was used as a control strain and untreated cultures of the respective isolates were used as growth controls. The microtiter plates were incubated at 35°C for 48 hours. Plates were read after 24 and 48 hours
STATISTICAL ANALYSIS	<ul style="list-style-type: none"> Descriptive characteristics of the study participants were presented by Candida status, as frequencies and percentages of the categorical variables. Comparisons by Candida status in the descriptive characteristics were performed using Chi square tests with a 5% significance level. P-values ≤0.05 were considered significant. All analyses were conducted using STATA

RESULTS

- Candida infection prevalence = 48.0% (72/150)
- candida *albicans* prevalence = 100.0% (72/72)
- The majority of the isolates (45/72; 62.5%) yielded a 450bp band which was assigned Genotype A. Of the 72 isolates, 19 isolates (26.4%) yielded a band size of 840bp and was assigned Genotype B. A total of 11.1% (8/72) of the isolates yielded band sizes of 450bp and 840bp which was Genotype C.

Table 2: Correlation between susceptibility profiles and genotypes

Genotypes	Susceptibility pattern	
	Susceptible (n= 15)	Resistant (n= 57)
A	9/45 (20%)	36/45 (80%)
B	5/19 (26.3)	14/19 (73.7%)
C	1/8 (12.5%)	7/8 (87.5%)

Table 1: Distribution of the different genotypes in association with clinical factors

CLINICAL FACTORS	GENOTYPES (n=72)		
	A (n=45)	B (n=19)	C (n=8)
Current abnormal vaginal discharge			
Yes	10 /17 (58.8%)	6/17 (35.3%)	1/17 (5.9%)
No	35/55 (63.6%)	13/55 (23.6%)	7/55 (12.7%)
Past treatment of STI			
Yes	25/39 (64.1%)	10/39 (25.6%)	4/39 (10.3%)
No	20/33 (60.6%)	9/33(27.3%)	4/33 (12.1%)
Past abnormal vaginal discharge			
Yes	25/40 (62.5%)	11/40 (27.5%)	4/40 (10%)
No	20/32 (62.5%)	8/32 (25%)	4/32 (12.5%)
Pregnancy status			
Pregnant	19/31 (61.3%)	8/31 (25.8%)	4/31 (12.9%)
Non-pregnant	26/41 (63.4%)	11/41 (26.8%)	4/41 (9.8%)
HIV status			
Positive	31/51 (60.8%)	13/51(25.5%)	7/51 (13.7%)
Negative	14/21 (66.7%)	6/21 (28.6%)	1/21 (4.8%)

DISCUSSION AND CONCLUSION

- Characterization of Candida isolates plays a critical role in determining antimicrobial susceptibility profiles and resistance mechanisms in vaginal Candida infections. Drug resistance leads to various treatment failures, compromising the patient's overall health (Liu et al., 2009).
- In the current study, the prevalence of vaginal Candida infection was 48.0%. This prevalence was higher when compared to the prevalence observed in Yemen, Nigeria and India where the prevalence of vaginal Candida infection was found to be 22.1%, 30.0% and 43.0% respectively (Okonkwo & Umeanaeto, 2010)(Nelson et al., 2013)(Kombade et al., 2021).
- In the current study (62.5%) of the isolates were assigned Genotype A, 26.4% were assigned Genotype B, and 11.1% of the isolates were assigned Genotype C. Our results corroborate with another study which found Genotype A to be the most prevalent genotype with a prevalence of 75.7% (Fornari et al., 2016).
- Antifungal susceptibility results showed that of the 72 isolates, 79.2% were resistant to amphotericin B and 20.8% were susceptible to amphotericin B. This study observed a high prevalence of resistance to amphotericin B
- When linking MIC patterns to distribution of genotypes, it was observed that the majority (80%) of the isolates which were assigned genotype A were resistant to amphotericin B. When linking clinical symptoms with the distribution of genotypes, it was observed that the majority (58.8%) of women who reported having current symptoms of abnormal vaginal discharge carried genotype A. Genotype A was most prevalent in women who had been treated for vaginal infections in the past and in women who were HIV positive with prevalence of 64.1% and 60.8%, respectively. genotype A was most prevalent in the non-pregnant women with a prevalence of 63.4%. Genotype A was prevalent (61.3%) amongst the pregnant women and the majority (66.7%) of the HIV negative women had Candida infections which belonged to genotype A.
- The prevalence of Candida was shown to be high in both pregnant and non-pregnant women in this study. This study also found a high level of resistance to the antifungal amphotericin B. Currently in our local setting, resistance patterns to the commonly used antifungals to treat Candida infections are not being monitored. There is a need for antifungal resistance monitoring in order to reduce the risk of future persistent and untreatable infections.