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Identification and Antifungal Susceptibility Profiles of Yeast Urogenital Tract Infection among Women in Benin City, Nigeria

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ABSTRACT: The incidence of candida infections is on the increase globally, with laboratories not performing speciation and susceptibility testing routinely. This study aims to speciate and determine the susceptibility profile of yeast isolates recovered from urogenital specimens. A total of 115 female participants with signs and symptoms of urogenital tracts infection attending various clinics in University of Benin Teaching Hospital Benin City, Nigeria were recruited for this study. A total of 55 mid-stream urine and 60 high vaginal swabs and or endocervical swabs were collected from the participants and processed to recover yeast isolates. The yeast isolates were identified and susceptibility test performed using standard techniques. The prevalence of candiduria - 54.55% and vaginal candidiasis - 63.33%, did not differ significantly (P = 0.4426). Age did not significantly affect the prevalence of candiduria and vaginal candidiasis (P = 0.0525 and P = 0.2194, respectively). *Candida albicans* was the most prevalent yeast isolate recovered from urine and genital specimens. Amphotericin B and nystatin were the most active antifungal agents while fluconazole was the least active. High prevalence of yeast urogenital tract infections was observed in this study.

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Keywords: Antifungal susceptibility; urogenital tract infection; endocervical swabs; candiduria; vaginal candidiasis

Female urogenital infections are infections that affect the bladder, kidneys vagina, cervix, urethra and periurethral (Edwards, 2004). These infections are either due to bacteria, yeast, viruses and parasites with the main clinical outcome being morbidity and discomfort among a large percentage of female population (Edwards, 2004). It is estimated annually that over one million women around the globe suffer from non–sexually transmitted urogenital infections caused by yeast and other agents (Durer *et al.*, 2003). The most common causes of urogenital yeast infections are *Candida* species (Richards *et al.*, 2000), which can lead to a wide range of life–threatening invasive to non–life threatening mucocutaneous disease (Sobel *et al.*, 2011). Amongst the species of candida that causes urogenital infections, *Candida albicans* is known to be the most common infectious agent (Jain *et al.*, 2011). *Candida albicans* is a commensal capable of colonizing the gastrointestinal tract and the reproductive tract, though the non – albicans group are beginning to emerge as pathogens in these mucocutaneous surfaces (Sobel., 2006). The most common manifestation of urogenital infections in women are the vulvovaginal candidiasis (VVC) and candiduria (Achkar and Fries, 2010). Vulvovaginal candidiasis affects mostly women of child bearing age while candidiasis is common among immuno-compromised patients (Sobel *et al.*, 1998). Ordinarily, the presence of candida in the vagina without any sign and symptoms is usually referred to a colonization and when the normal conditions are altered due to underlying ailments, incessant and uncontrolled use of antimicrobial agents, the normal pH of the vaginal is altered, these can make the urogenital tracts susceptible to urogenital candidiasis (Alvarez–Lerma *et al.*, 2003; Pappas *et al.*, 2009). The polyenes and the azoles are the most commonly used antifungal agents for the treatment of urogenital infections, although resistance is beginning to emerge in different areas most especially with the non–albicans candida that are intrinsically resistant to antifungal agents (Achkar and Fries, 2010; Manjunath *et al.*, 2011). Fungal resistance

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is responsible for persistent infections (Manjunath *et al.,* 2011). However, speciation and susceptibility test for yeasts are not routinely done in our environment this may result in the poor management of yeast urogenital tract infection. Against this background, this study is aimed to identification and antifungal susceptibility profiles of yeast urogenital tract infection among women in Benin City, Nigeria

MATERIALS AND METHODS

Study population: This study was carried out in the University of Benin Teaching Hospital Benin City, Nigeria. A total of 115 female patients with signs and symptoms of urogenital tract infection were recruited for this study. Informed consent was obtained from each patient prior to specimen collection. The Ethics and Research Committee of the University of Benin Teaching Hospital approved the protocol for the study.

*Collection and processing of specimens***:** A total of 60 High vaginal swab (HVS) and endocervical swab (ECS) were collected from women with signs and symptoms of genital conditions while 55 mid-stream urine samples were collected from women with signs and symptoms of urinary tract infection. The patients from which the genital specimens were collected were different from the patient that produced the urine specimens.

Processing of specimens: HVS /ECS: A modification of the method described by Chessbrough (2004), was used to process the HVS/ECS swabs. Briefly, two swabs were collected from each specimen. One of the swabs was inoculated onto sabouraud dextrose agar containing 20µg/ml of gentamicin and the plates were incubated at 37^0C aerobically for 24 – 48hrs. Films were made from the other swab and stained by Grams staining method, and for wet preparation. Briefly, 2ml of normal saline was added to the swab and mixed, this was allowed to stand for about 30 minutes and a drop of this preparation was placed on a slide and covered with a coverslip this was examined under X10 and X40 objective for yeast cells.

Urine: The urine specimens were processed as described by Esebelahie et al (2013a). Briefly, a loopfull (0.001m) of well mixed uncentrifuged urine was streaked onto the surface of sabouraud dextrose agar containing 20µg/ml of gentamicin. The plates were incubated aerobically at 37°C for 24 – 48hrs and counts expressed in cfu/ml. A count of $\geq 10^5$ cfu/ml was considered indicative of urinary tract infections. The urine specimens were centrifuged at 2000g for 5minutes. The supernatant was discarded and a loop of the deposit was examined microscopically at high magnification for pus cells, yeast cells, red blood cells and *Trichomonans vaginalis.* Pus cells ≤5 per high power field was considered indicative of infection. Urinary candidiasis was diagnosed if the yeast of pus cells count or both were significant in an individual.

Identification and speciation of isolates: Yeasts isolates were identified by Germ tube and chlamydiospore formation, sugar fermentation and assimilation tests as previously described (Amar *et al.,* 2013), as well as CHROMagar candida (CHROMagar, Paris, France) (Paripokee)

Antifungal disc susceptibility testing: Disc antifungal susceptibility testing was performed on the yeast isolates using the agar diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2009). The antifungal agents used were fluconazole (25µg), ketoconazole (10µg), Amphotericin B (25µg) and nystatin (100 units).

Statistical analysis: The data obtained were analysed with Chi square (X^2) test using the statistical software INSTAT $^{(R)}$ (Graph Pad software Inc. La Jolla, Ca, USA).

RESULTS AND DISCUSSION

The prevalence of candiduria and genital candidiasis in this study was 54.55% and 63.33%, respectively. The prevalence of candiduria and gential candidiasis did not differ significantly (P = 0.4426), as well as in relation to age (P > 0.05) (Table 1). *Candida albicans* were the predominant species recovered generally and from both urine and genital specimens. Generally, *Candida glabrata* and *Candida parasilopsis* were the least prevalent yeast isolates with a prevalence of 1.43% and were not recovered from genital tract specimens (Table 2). Susceptibility profile of the antifungal agents that were used on the urine isolates showed that the polyenes (Amphotericin B and Nystatin) were 100% active against all yeast isolates. Fluconazole and ketoconazole were not active against *Candida tropicalis, Cryptococcus neoformans* and *Candida krusei* (Table 3). Similarly, yeasts isolate from genital specimens were susceptible to the polyenes, while the azole were less susceptibile against the *Candida albicans* and fluconazole was not active against *Candida lusitanea* that were recovered from the genital specimens.

The incidence of candida has been on the rise worldwide (Goyal *et al.,* 2016). In general, routine laboratories do not perform identification and cultivation of yeast (Sousa *et al.,* 2014). Against this background, this study was conducted. The prevalence of candiduria observed in this study was 54.55%. This is higher than the 5.38% and 2.36% reported (Goyal *et al.,* 2016; MahanJan *et al.,* 2015), respectively. The difference may be due to geographical location and the diagnostic methods used. The studies were carried out in India, while our study was done in Nigeria. In both studies (Goyal *et al.,* 2016; MahanJan *et al.,* 2015), the

urine specimens were plated on either blood agar, MacConkay and or cystein lactose electrolyte deficient (CLED) medium in our study the urine specimens were plated directly on Sabouraud dextrose agar containing 20µg/ml gentamicin. The use of sabouraud dextrose agar have been reported to detect higher number of *Candida* species compared with media used for standard urine bacteriology (Achkar and Fries, 2010; Jain *et al.,* 2007; Okuliez *et al.,* 2008).

Table 1: Prevalence of yeast infections recovered from female urogenital specimens

Characteristics	No. tested	No. infected (%)	P- value
Specimens			
Urine	55	30(54.55)	0.4426
Genital	60	38(63.33)	
Age (year)			
Urine			
≤ 21 – 30	10	2(20.00)	0.0525
31 – 40	5	3(60.00)	
≥ 41	40	25(62.50)	
Genital			
≤ 21 – 30	10	8(80.00)	0.2194
31 – 40	6	5(83.33)	
≥ 41	44	25(56.82)	

Genital specimens include high vaginal and endo-cervical swabs

Table 2: Distribution of yeast species recovered from female uro-genital specimens

Yeast isolates	Urine %	Genital %	Total %
Candida albicans	15(60.00)	30(76.92)	45(62.29)
Candida krusei	1(4.00)	3(7.69)	4(5.71)
Candida glabrata	1(4.00)	0	1(1.43)
Candida lusitanea	6(24.00)	3(7.69)	9(12.86)
Candida parasiplosis	1(4.00)	0	1(1.43)
Candida tropicalis	4(16.00)	1(2.56)	5(7.14)
Cryptococcus neoformans	1(4.00)	1(2.56)	2(2.86)
Saccharomyces cerevisiae	2(8.00)	1(2.56)	3(4.29)
Total	25	39	70

Table 3: Susceptibility profiles of yeast isolates recovered from urine specimens.

	Antifungal agents			
Susceptibility	**FCN (25µg)**	**KCN(10µg)**	**Amp B (25µg)**	**NYS(100unit)**
Candida albicans (n = 15)	12(80.00)	12(80.00)	15(100.00)	15(100.00)
Candida glabrata (n = 1)	1(100.00)	1(100.00)	1(100.00)	1(100.00)
Candida krusei (n = 1)	0(0.00)	1(100.00)	1(100.00)	1(100.00)
Candida lusitanea (n = 6)	1(16.00)	5(83.00)	6(100.00)	6(100.00)
Candida parasilopsis (n = 1)	1(100.00)	1(100.00)	1(100.00)	1(100.00)
Candida tropicalis (n = 4)	0 (0.00)	1(25.00)	4(100.00)	4(100.00)
Cryptococcus neoformans (n = 1)	0(0.00)	1(100.00)	1(100.00)	1(100.00)
Saccharomyces cerevisiae (n = 2)	1(50.00)	2(100.00)	2(100.00)	2(100.00)

FCN = fluconazole, KCN= ketoconazole, Amp B = Amphotericin B and NYS = Nystatin

Table 4: Susceptibility profiles of yeast isolates recovered from genital specimens

	Antifungal agents			
Susceptibility	**FCN (25µg)**	**KCN (10µg)**	**AmpB(25µg)**	**NYS (100unit)**
Candida albicans (n = 30)	10(33.33)	14(46.67)	22(73.33)	22(73.33)
Candida krusei (n = 3)	2(66.67)	2(66.67)	2(66.67)	3(100.00)
Candida lusitanea (n = 6)	0	2(66.67)	2(66.67)	2(66.67)
Candida tropicalis (n = 1)	1(100.00)	1(100.00)	1(100.00)	1(100.00)
Cryptococcus neoformans (n = 1)	1(100.00)	1(100.00)	1(100.00)	1(100.00)
Saccharomyces cerevisiae (n = 1)	1(100.00)	1(100.00)	1(100.00)	1(100.00)

Genital specimens include high vaginal and endo-cervical swabs, FCN=fluconazole, KCN=ketoconazole, AmpB=AmphotericinB and NYS=Nystatin

This may explain the findings in this study. A prevalence of genital candidasis of 63.33% was observed in this study, this is higher than the 55% and 56% reported from two studies in United States of America (Chessbrough, 2004; Geiger *et al.,* 1995). Although Achkar and Fries (2010) reported no data on the incidence rate of vulvovaginal candidiasis in relation to race (Foxman, 1990; Geiger *et al.,* 1995), reported higher prevalence of vulvovaginal candidiasis among African-Americans than white woman or woman of other races. Our study participants are all Africans. This may explain the higher prevalence observed in this study. However, a prevalence of 29.1% of vulvovaginal candidiasis was reported in Jos, Nigeria (Jumbo *et al.,* 2010). The reason for this is unclear. There was no significant difference (P = 0.4426) in the prevalence of urinary candidiasis and genital candidiasis in this study.

Although, the patients from which urine and genital tract specimens were collected from in this study are different, it has been reported that Candida present in the genital tract of the female, ascend the short female urinary tract to cause urinary candidiasis (Achkar and Fries, 2010). This may explain the finding in this study. The finding that *Candida albicans* was the most predominant yeast recovered from urogenital specimens agrees with the previous reports (Esebelahie *et al.,* 2013a; Esebelahie *et al.,* 2013b). The finding of *Cryptococcus neoformans* in urogenital specimens agrees with previous reports (Dzoyem *et al.,* 2010; Lungran *et al.,* 2014). Similarly, the finding of *Saccharomyces cerevisiae* in urogential tracts specimens is in agreement with previous findings (Enache–Angoulvant and Hennequin, 2005). Other yeast isolates recovered in this study have been reported to be previously recovered from the urogenital tract specimens (Sousa *et al.,* 2014). Generally, yeast isolates from urine were more susceptible to polyenes than those from the vaginal. The reason could be the indiscriminate use of various antifungal agents especially as suppositories to treat yeast infections. A similar picture was observed for fluconazole against *Candida albicans, Candida tropicalis* and *Cryptococcus* neoformans recovered from urine were not susceptible to fluconazole as against the 100% susceptibility of the isolates from the genital tract. The polyenes were the most active antifungal agents against yeast isolates recovered from the urogenital tracts, while the activity of the azoles vary depending on the yeast isolates and the site of recovery.

Conclusion: This study reveals high prevalence urogenital tracts infections which were not influenced by age. *Candida albicans* was the most prevalent yeast recovered and the polyenes were the most active antifungal agents. Prudent use of antifungal agents is advocated.

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