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

OLADUGBA, EO

Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

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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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 (Álvarez–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

*Corresponding Author Email: omuaevelyn@gmail.com
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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°C 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 (0.001ml) 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 ≥ 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 genital 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 polynes (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 polynes, while the azole were less susceptible against the Candida albicans and fluconazole was not active against Candida lusitaniae 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
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urine specimens were plated on either blood agar, MacConkey 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).

This may explain the findings in this study. A prevalence of genital candidiasis 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.

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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)      |            |                  |         |
| ≤ 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 urogenital specimens

| Yeast isolates          | Genital % | Urine % | 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(0.00) | 1(1.33) |
| Candida lusitanae       | 6(24.00)  | 3(7.69) | 9(12.86)|
| Candida parapsilosis    | 1(4.00)   | 0(0.00) | 1(1.33) |
| 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                   | 12(80.00) | 15(100.00)| 27(36.57)|

Table 3: Antifungal agents

| Antifungal agents | FCN (25µg) | KCN(10µg) | Amp B (25µg) | NYS(100unit) |
|-------------------|------------|-----------|--------------|--------------|
| Candida albicans  | 12(80.00)  | 12(80.00) | 15(100.00)   | 15(100.00)   |
| Candida glabrata  | 1(100.00)  | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Candida krusei    | 0(0.00)    | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Candida lusitanae | 1(16.00)   | 5(83.33)  | 6(100.00)    | 6(100.00)    |
| Candida parapsilosis | 1(100.00) | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Candida tropicalis| 0(0.00)    | 1(25.00)  | 4(100.00)    | 4(100.00)    |
| Cryptococcus neoformans | 0(0.00) | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Saccharomyces cerevisiae | 1(50.00) | 2(100.00) | 2(100.00)    | 2(100.00)    |
| Total             | 15(100.00) | 15(100.00) | 15(100.00)   | 15(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 | FCN (25µg) | KCN(10µg) | Amp B (25µg) | NYS(100unit) |
|-------------------|------------|-----------|--------------|--------------|
| Candida albicans  | 10(33.33)  | 14(46.67) | 22(73.33)    | 22(73.33)    |
| Candida krusei    | 2(66.67)   | 2(66.67)  | 2(66.67)     | 3(100.00)    |
| Candida lusitanae | 0          | 2(66.67)  | 2(66.67)     | 2(66.67)     |
| Candida tropicalis| 1(100.00)  | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Cryptococcus neoformans | 1(100.00) | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Saccharomyces cerevisiae | 1(100.00) | 1(100.00) | 1(100.00)    | 1(100.00)    |
| Total             | 18(60.00)  | 16(53.33) | 18(60.00)    | 18(60.00)    |

Genital specimens include high vaginal and endo-cervical swabs, FCN=fluconazole, KCN=ketoconazole, AmpB=Amphotericin B and NYS=Nystatin
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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 urogenital 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.

**REFERENCES**

Achkar, JM; Fries, BC (2010). Candida infections of the genitourinary tract. *Clin Microbiol Rev*, 23(2): 253-273.

Álvarez-Lerma, F; Nolla-Salas, J; León, C; Palomar, M; Jordá, R; Carrasco, N; Bobillo, F (2003). *Candiduria* in critically ill patients admitted to intensive care medical units. *Intens Care Med*, 29(7): 1069-1076.

Amar, CS; Ashish, J; Hajare, V; Yogesh, B (2013). Study of prevalence and antifungal susceptibility of candida. *Int J Pharma and Bio Sci*, 4(2).

Cheesbrough, M (2005). *District laboratory practice in tropical countries*, part 2. Cambridge university press.

Clinical Laboratory Standards Institute (2009). Method of antifungal disc susceptibility testing of yeast; approved guidelines – second edition. C.L.S.I. document M 44 – A2 (ISBNI – 56238 – 703 – 0). Clinical Laboratory Standard Institute, Wayne: Pennsylvania.

Duerr, A; Heilig, CM; Meikle, SF; Cu-Uvin, S; Klein, RS; Rompalo, A; HER Study Group (2003). Incident and persistent vulvovaginal candidiasis among human immunodeficiency virus–infected women: Risk factors and severity. *Obstet & Gynecol*, 101(3): 548-556.

Dzoyem, JF; Kechia, FA; Ngaba, GP; Lunga, PK; Lohoue, PJ (2012). Prevalence of cryptococcosis among HIV-infected patients in Yaounde, Cameroon. *Afri Health Sci*, 12(2): 129-133.

Edwards, L (2004). The diagnosis and treatment of infectious vaginitis. *Dermatol Ther*, 17(1): 102-110.

Enache-Angoulvant, A; Hennequin, C (2005). Invasive *Saccharomyces* infection: a comprehensive review. *Clin Infect Dis*, 41(11): 1559-1568.

Esebelahie, NO; Ewaeni, IB; Omorogie, R (2013). Candida colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. *Liby J Med*, 8(1).

Esebelahie, NO; Ewaeni, IB; Newton–Esebelahie, FO; Omorogie, R (2013). A symptomatic oral yeast – carriage among HIV and Non HIV individual in Benin City, Nigeria. *Int Arch Microbiol & Immunol*, 1(1): 1–5.

Foxman, B. (1990). The epidemiology of vulvovaginal candidiasis: risk factors. *Amer J Publ Health*, 80(3): 329-331.

Geiger, AM; Foxman, B; Gillespie, BW (1995). The epidemiology of vulvovaginal candidiasis among university students. *Amer J Publ Health*, 85(8_Pt_1): 1146-1148.

Goyal, RK; Sami, H; Mishra, V; Bareja, R; Behara, RN (2016). Non-albicans candiduria: an emerging threat. *J Appl. Pharmaceut. Sci.*, 6(3): 048-050.
Identification and antifungal susceptibility profiles of yeast….

Jain, M; Dogra, V; Mishra, B; Thakur, A; Loomba, PS; Bhargava, A (2011). Candiduria in catheterized intensive care unit patients: emerging microbiological trends. Ind J Pathol and Microbiol, 54(3): 552.

Jain, N; Kohli, R; Cook, E; Gialanella, P; Chang, T; Fries, BC (2007). Biofilm formation by and antifungal susceptibility of Candida isolates from urine. Appl & Environ Microbiol, 73(6): 1697-1703.

Jombo, GTA; Opajobi, SO; Egah, DZ; Banwat, EB; Akaa, PD (2010). Symptomatic vulvovaginal candidiasis and genital colonization by Candida species in Nigeria. J Publ Health Epidemiol, 2(6): 147-151.

Lungran, P; Vijaya, D; Shashi, S (2014). Cryptococcosis: its prevalence and clinical presentation among HIV positive and negative patients in Rims, Manipur. IOSR J Dent & Med Sci, 13(7): 38.

MahanJan, A; Kaur, A; Singh, S; Gill, A (2015). Isolation, identification and antifungal susceptibility pattern of Candida species isolated from UTI cases in Tertiary care Hospital. Schol J Appl Med Sci. 3 (5E): 2146 – 2152.

Manjunath, GN; Prakash, R; Vamseedhar AKS (2011). Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore. Int J Biol Med Res., 2(2), 504-507.

Okuliez, JR; Rivard, RG; Conger, NG; Ngugen, MX; Hospenthal, DR (2008). Primary isolation of candida from urine specimen using chromogenic medium. Mycosis. 51: 141 – 146.

Pappas, PG; Kauffman, CA; Andes, D; Benjamin Jr, DK; Calandra, TF; Edwards Jr, JE; Sobel, JD (2009). Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clinical infectious diseases: an official publication of the Infect Dis Soc Ame, 48(5): 503.

Paritpokee, S; Hall, G; Procop, G (2005). Rapid identification of yeast isolates using BD BBL™ CHROMagar™ Candida. In 105th Gen Meet Amer Soc for Microbiol.

Richards, MJ; Edwards, JR; Culver, DH; Gaynes, RP; National Nosocomial Infections Surveillance System (2000). Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Contr & Hosp Epidemiol, 21(8): 510-515.

Sobel, JD (2006). The emergence of non-albicans Candida species as causes of invasive candidiasis and candidemia. Curr Infect Dis Report, 8(6): 427-433.

Sobel, JD; Faro, S; Force, RW; Foxman, B; Ledger, WJ; Nyirjesy, PR; Summers, PR (1998). Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Ame J Obstetr & Gynecol, 178(2): 203-211.

Sobel, JD; Fisher, JF; Kautfman, CA; Newman, CA (2011). Candida urinary tract infection epidemiology. Clin Infect Dis. 6: 5433–5432.

Sousa, IAD; Braoios, A; Santos, TG; Lima, JAD; Costa, RMD (2014). Candiduria in adults and children: prevalence and antifungal susceptibility in outpatient of Jatai-GO. J Bras de Patol e Med Lab. 50: 259-264.

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