

UROPATHOGENIC AND ANTIBIOTIC RESISTANCE OF *CANDIDA* SPECIES AMONG WOMEN VISITING A TERTIARY CARE HOSPITAL IN GABORONE, BOTSWANA

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ABSTRACT

The present paper aims to determine the prevalence and antibiotic susceptibility of *Candida* uropathogens among women at a tertiary hospital in Gaborone, Botswana. A total of 180 urine samples were collected from females of different age groups with UTIs from Princess Marina Hospital, Gaborone and assayed for the presence of *Candida* species. Five species of *Candida*, namely *C. albicans*, *C. glabrata*, *C. krusei*, *C. dubliniensis* and *C. tropicalis* were tentatively identified and antibiotic susceptibility was carried out on chromogenic media using the Vitek 2 system. The identification of these species were confirmed by amplifying the internal transcribed spacer regions (ITS). To amplify the superseding 5.8S ribosomal Deoxyribonucleic Acid (rDNA) and the adjacent ITS1 and ITS2 regions, the ITS1-ITS4 primer pair was used. Candidal uropathogens accounted for 38.8 % of UTIs out of 180 urine samples. *Candida albicans* was the most predominant in the urine samples accounting for 45.7 % followed by *Candida glabrata* (17.1 %), while *C. krusei* and *C. dubliniensis* each accounted for 10 % and *Candida tropicalis* 5.5%. There was no statistically significant difference ($p < 0.05$) between the rate of isolation of *Candida* spp. Although a high prevalence of candiduria (51.4%) was associated with females in the 26-35 age bracket, this effect was not statistically significant ($p < 0.05$) among different age groups. Three non-*Candida* species (1.6%), *Cryptococcus neoformans*, *C. albii* and *Rhodotorula* sp. were also detected from urine samples. This finding is alarming and demonstrates that these species may emerge as opportunistic pathogens in UTIs. *C. dubliniensis* was highly resistant to flucytosine (100%) and fluconazole (60%). The results of the present study highlight a growing problem of antifungal resistance among some *Candida* uropathogens and call for a need to establish stringent measures in monitoring the same.

Keywords: *Candida*, Urinary Tract Infections (UTIs), Antibiotic Resistance, Internal Transcribed Spacer Region (ITS)

INTRODUCTION

Urinary tract infections (UTIs) are the most common infections that afflict humans in the community and hospital settings, and often result in increased rates of morbidity and high economic costs associated with their treatment (Foxman, 2002; Shah *et al.*, 2015; Preesthishree *et al.*, 2016). Underlying host factors such as gender, age, urogenital dysfunction, immunosuppression, indwelling catheters and sexual behaviour have been shown to predispose individuals to urinary tract infections (Dwyer and O'Reilly, 2002).

Various studies (Stamm, 2001; Nimri and Batchoun, 2010) have shown that women are more likely to experience UTIs than men. This mainly owes to the urethra of women being shorter and in closer proximity to the anus than in men, hence allowing pathogens to gain quicker access to the bladder. It is estimated that 20% of women experience urinary tract infections and the disease is especially more common among the elderly and pregnant women (Dwyer and O'Reilly, 2002).

Although *Escherichia coli* tends to be the most predominant microorganism associated with urinary tract infections (Orhue, 2014), *Candida* spp. have in the past few decades come to the fore as significant causes of UTIs (Brito *et al.*, 2006; Kaplana *et al.*, 2015). They are opportunistic microorganisms which are found as normal flora in the female genital area (Payam *et al.*, 2010), and have virulence factors such as secreted aspartyl proteinases (SAPs) and phospholipases which facilitate host tissue adhesion and invasion (Naglik *et al.*, 2005; Birinci *et al.*, 2006).

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There are eight opportunistic *Candida* spp. namely; *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. dublineinsis* and *C. tropicalis* (Anaissie et al., 2003). Urinary tract infections due to *Candida* species are known as candiduria and *C. albicans* has been found be the most prevalent cause of these infections, accounting for about 50% of the cases of candiduria (Alenzi, 2016). *Candida* infections are routinely treated with antifungals such as Itranacazole (De Backer et al., 2001) and Flucytosine (Sobel et al., 2003). However, a growing problem associated with clinical *Candida* isolates is antifungal resistance, where the species have been found to employ resistance mechanisms like biofilm formation (Douglas, 2003) and efflux pumps (Ramage et al., 2002).

Advanced molecular identification approaches of yeast species are based on polymerase chain reaction (PCR), that is, PCR-RFLP, real-time PCR which overcome the limitations of phenotypic methods of yeast identification (Hierro et al., 2004). Between coding regions of the 18S, 5.8S, and 28S nuclear rRNA genes are the internal transcribed spacer 1 and 2 regions (ITS1 and ITS2, respectively) which evolve more rapidly and may therefore, vary among different species within a genus (Chen et al., 2000). In this regard, PCR amplification of ITS region DNA and sequencing them stands out to be the most appropriate method used for identifying fungal species.

In Botswana, *Candida* spp. have not only been isolated from different food products such as vegetables and biltong (Manani et al., 2006; Matsheka et al., 2014) but have also been found to account for up to 49% of pathogens in women with vaginal discharge (Paz-Bailey et al., 2006). Urinary tract infections (UTIs) and increasing antibiotic resistance of candida uropathogens are a global concern particularly in immunocompromised patients. However, no study has been done in Botswana addressing the role of *Candida* spp. in urinary tract infections in women, and their identification using molecular tools. Hence, the objective of the present study was to identify and determine the prevalence and antibiotic resistance of *Candida* uropathogens among women at a tertiary hospital in Gaborone, Botswana.

MATERIALS AND METHODS

Sample Collection and Processing

Early morning mid-stream urine samples were collected in sterile containers from 180 women diagnosed as suffering from urinary tract infections from November 2013 to April 2014 at Princess Marina Hospital, a tertiary hospital in Gaborone Botswana. Upon collection, the color of the urine was noted and then samples transported aseptically in a cooler box to the Microbiology laboratory of the Department of Biological Sciences, University of Botswana.

Screening and Culturing of Candida Isolates

On arrival at the laboratory, the Combur 10 Test® (Roche Diagnostics GmbH, Mannheim, Germany) was used to detect leukocytes in the urine which served as an indirect indicator of urinary tract infections. Isolates that tested positive for leukocytes were spun at medium speed in a centrifuge (Z 233 M-2, Hermle Labortechnik GmbH, Germany). The supernatant was discarded and a wet mount prepared and examined microscopically. Candiduria was confirmed by isolation of one or two *Candida* sp. with less than 10^5 colony forming units per milliliter and presence of pyuria (>6 pus cells per high power field). Using a calibrated loop of 3mm diameter, appropriately labeled CHROMagar Candida (CHROMagar Company, Paris, France) plates were streaked aseptically with 0.01 ml urine sample. The plates were then incubated at 37°C for 24 hours and thereafter observed for characteristic colors displayed by different *Candida* species.

Identification and Antibiotic Resistance Testing of Yeast Isolates

Identification and antibiotic resistance testing of the tentative *Candida* isolates on chromogenic media was carried out using the Vitek 2 system (BioMerieuxVitek. Inc. Hazelwood, MO) following the manufacturer's instructions. The antifungals evaluated were amphotericin B, caspofungin, fluconazole, flucytosine, and voriconazole.

DNA Extraction and Amplification of the Internal Transcribed Spacer Region

Candida isolates were sub cultured on Sabouraud dextrose agar (Merck KGaA, Darmstadt, Germany) and CHROMagar Candida (CHROMagar Company, Paris, France) and incubated at 30°C for 48 h. Genomic

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DNA of each fresh colony was extracted using ZRFungal/Bacterial DNA MiniprepTM (Zymo Research., Irvine, CA, USA) according to the manufacturer's instructions.

The ITS gene region was amplified in a GeneAMP PCR system (Applied Biosystems, Carlifornia, USA). The primer pair used in the amplification of the ITS region, ITS1 (5'-TCCGTTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), have been described previously (White *et al.*, 1990). Each PCR reaction mixture consisted of 12.5µl of 2X Master Mix (New England Biolabs, Ipswich, MA, USA), 1µl each of reverse and forward primers, 2µl of genomic DNA and the mixture made up to 25µl with sterile nuclease-free water.

The PCR was run with the following thermocycling conditions: initial denaturation at 94°C for 8 min; followed by 35 cycles for 1 min at 94°C, 55°C for 1 min and 2 min at 72°C and final extension at 72°C for 12min.

Three micro liter of the PCR products were resolved on 1.2% agarose gel (Sigma Aldrich, Missouri, USA) in TBE buffer (Tris 90mM, Boric acid 90mM, EDTA 2mM), stained with 0.5 µg/ml of ethidium bromide.

The gels were run for 45 minutes at 80 volts and gels were then visualized on a gel documentation system (Bio-Rad, Carlifornia, USA).

Data Analysis

Graphpad Prism 7 (GraphPad Software Company, LaJolla, CA, USA) was used to analyze the statistical significance of the data.

One-way ANOVA was employed to separate the means of occurrence of *Candida* spp. in different urine samples investigated in this study. Fisher's exact tests were used to analyze the frequency of *Candida* spp. in urine samples.

RESULTS AND DISCUSSION

Results

Out of the 180 urine samples, a total of 60 (38.8 %) tested positive for *Candida* spp. The most predominant *Candida* spp. in the samples isolated was *Candida albicans* with a prevalence of 45.7 % (Figure 2).

The next most prevalent was *Candida glabrata* followed by *Candida krusei*, *Candida dublineinsis* and *Candida tropicalis* with a prevalence of 17.1%, 14.2%, 10% and 10 % respectively. Three (3) isolates (1.6%) were of non-*Candida* species namely; *Cryptococcus albius*, *Cryptococcus neoformans* and *Rhodotorula* species. There was no statistically significant difference ($p < 0.05$) on the isolation rate of *Candida* spp. from urine samples.

A comparison of the occurrence of different *Candida* spp species in urine samples showed that their rate of occurrence was not statistically significant ($p < 0.05$).

PCR amplification using universal fungal primers (ITS1/ITS4) yielded fragments of about 450-850 bp long (Figure 1). The sizes of the PCR products were nearly equal in size for some species; *C. tropicalis* 530, *C. albicans* 550 and *C. krusie* 510.

However, all yeast isolates showed species-specific differences in the sizes of the PCR products amplified. *C. glabrata* had the largest fragment size of about 780 bp.

Figure 1 shows the distribution of *Candida* spp. and candiduria among different age groups of women. *C. albicans* was found to be the most dominant cause of candiduria in all age groups whereas other *Candida* spp. were mostly associated with females in the age bracket 26-35.

C. glabrata and *C. krusei* were equally the second most common cause of candiduria in females in age group 26-35. *C. glabrata*, *C. dubliniensis* and *C. tropicalis* were the second most common cause of candiduria in females in the age group 19-25.

A high rate of *Candida* infection was recorded among the age group 26-35 years (51.4%), the age group 46 and above had the least incidence of *Candida* uropathogens (2.9%). The age group 19-25 and 36-45 had an incidence of 34.2 % and 11.4 % respectively. There was no statistical significance ($p < 0.05$) in the prevalence of *Candida* spp. among all age groups.

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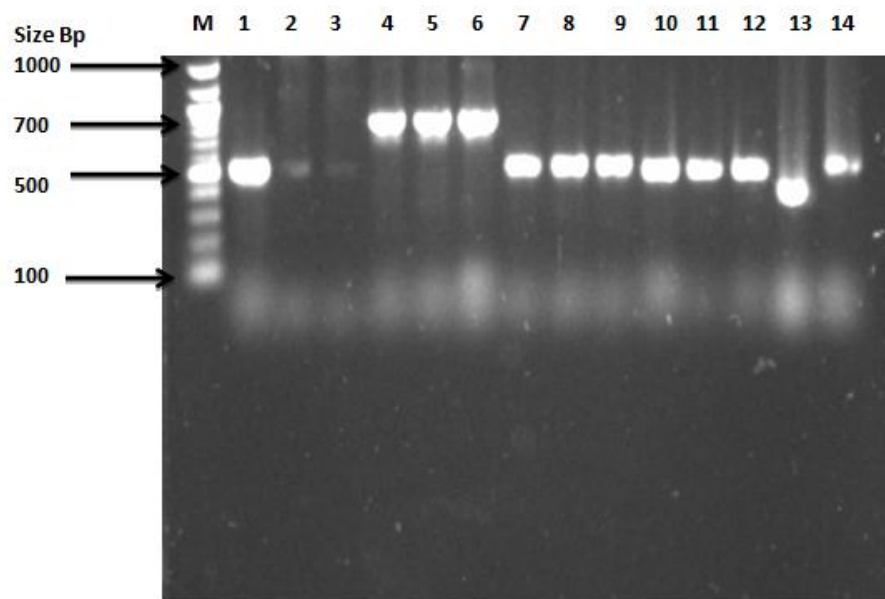


Figure 1: Gel Electrophoresis of the Amplified rDNA Internal Transcribed Sequence (ITS) Region of *Candida* Isolates with ITS1 and ITS4 Primers Pairs; (M) 100 bp DNA Ladder (New England Biolabs). *Candida Tropicalis* (Lanes 1-3), *C. Glabrata* (Lanes 4-6), *C. Albicans* (Lanes 7-9), and *C. krusei* (Lanes 10-12); *C. Lusitaniae* ATCC 34449 (Lane 13) and *C. Albicans* ATCC 10231 (Lane 14) Served as Positive Controls

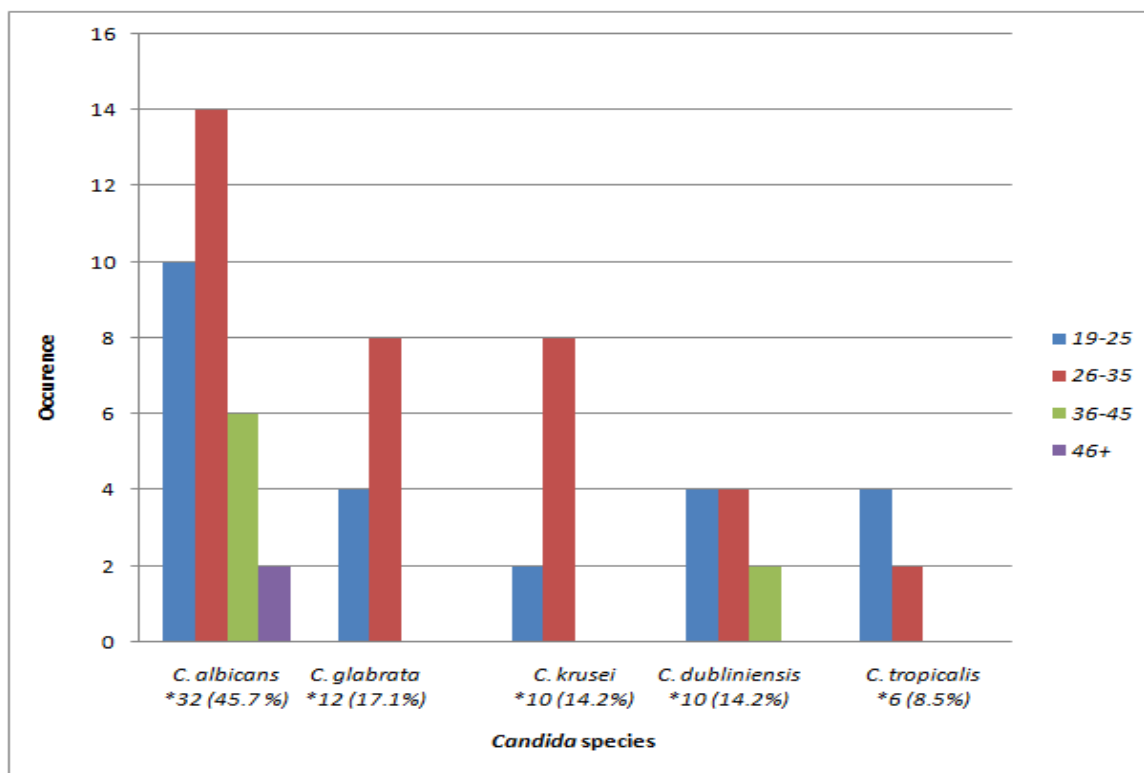


Figure 2: Distribution of Different *Candida* spp. within Different Age Groups of Women
 *Total Number and percentage of *Candida* species isolated from urine samples.

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Table 1 shows the resistance pattern of *Candida* spp. *C. albicans* and *C. dublineinsis* showed resistance of 18.8% and 100 % to flucytosine respectively. *C. glabrata* and *C. tropicalis* was susceptible to all antifungal agents tested against them. *C. dublineinsis* was resistant to three out five antifungal agents used in this study. Both antifungals amphotericin B and caspofugin each showed 100% of activity against all yeast tested in this study. There was no statistical significance between the activities of the tested drugs ($p < 0.05$).

Table 1: Number and Percentage of Candida Uropathogens Showing Antifungal Resistance Using Vitek 2 System

| Candida Species | Number of Isolates | Antifungal Agent | | | | |
|------------------------|--------------------|------------------|-------------|--------------|----------------|------------|
| | | Flucytosine | Fluconazole | Voriconazole | Amphotericin B | Caspofugin |
| <i>C. albicans</i> | 32 | 6 (18.8%) | 0 | 0 | 0 | 0 |
| <i>C. glabrata</i> | 12 | 0 | 0 | 0 | 0 | 0 |
| <i>C. dublineinsis</i> | 10 | 10 (100%) | 6 (60%) | 2 (20%) | 0 | 0 |
| <i>C. tropicalis</i> | 6 | 0 | 0 | 0 | 0 | 0 |
| Total | 60 | 16 (26.7%) | 6 (10%) | 2 (3.33%) | 0 | 0 |

Discussion

The present study utilized the biochemically-based Vitek 2 identification system and ITS PCR to differentiate *Candida* species among women with urinary tract infections at a tertiary hospital in Botswana. PCR amplification with specific primers for the ITS region generated bands ranging from 450 to 780 base pairs (Figure 1), with clear differences in fragment sizes among different species of *Candida* spp. analyzed. These findings are consistent with other studies (Fujita *et al.*, 2001; Ferrer *et al.*, 2001) which found different fragment sizes of PCR products when using ITS1 and ITS 4 primer pairs. However, some non-*Candida* yeast species (*Cryptococcus neoformans*, *Cryptococcus albus* and *Rhodotorula* sp) were also detected from urine samples. These species may be responsible for some cases of UTIs. Although this investigation focused on candiduria, this finding is alarming and demonstrates how other non-*Candida* yeast species may emerge as opportunistic pathogens creating increased healthcare burden especially in instances where HIV/AIDS continues unabated as is the case in Botswana.

A total of 60 (38.8 %) *Candida* spp. were isolated from urine samples among women diagnosed with UTIs, with *Candida albicans* being the most predominant species (45.7%). The prevalence rate of *Candida albicans* reported here is comparable to findings of 30% reported by Debora *et al.*, (2007) in Brazil. However, much lower rates of 10.2% and 8% were reported in Pakistan (Bagai *et al.*, 2008) and Saudi Arabia (El Sheikh *et al.*, 2000), respectively. Similarly, Tortorano *et al.*, (2006) found more than half of cases of candiduria to be accounted for by *Candida albicans*, followed by *C. glabrata* (14%) and *C. krusei* comprising only 2%. In contrast to this study, *C. tropicalis* was found to be the most predominant isolate from clinical samples, accounting for 54.5% prevalence of all isolates (Yesudhasan and Mohanra, 2015). The recent upsurge in UTIs due to *Candida* spp. is not completely understood but recent increases in such factors as prophylaxis by antifungal agents, catheterization, and uncontrolled use of antifungals have also seen corresponding increased cases of candiduria (Behzadi *et al.*, 2010). This study did not aim to investigate the factors that predispose women to candiduria, with age being the only demographic taken for the particular study population.

The highest proportion of *Candida* urinary tract infections was detected among women in the age bracket 19-35, with those above the age of 36 comparatively lower incidence rates. It is important to note that women in the former age bracket are more sexually active, and sexual activity is a well established factor that predisposes individuals to urinary tract infections (Ramesh and Agarwal, 2012). Some of the women with candiduria in this study were also pregnant and pregnancy has also been shown to predispose women

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to UTIs (Haider *et al.*, 2010). These two predisposing factors may be responsible for higher prevalence of candiduria in the 19-35 age bracket. The unprecedented levels of candiduria among women in this age bracket may also be exacerbated by HIV/AIDS infections in Botswana, which the Botswana AIDS Impact Survey in 2013 (Statistics Botswana, 2014) estimated to be 18.5%. Other studies also showed a higher frequency of UTI among HIV patients (Ibadin *et al.*, 2006).

The emergence of drug resistant *Candida* spp. which is largely attributed to use of prolonged and inappropriate empirical therapy, has further complicated patient management (Pfaller *et al.*, 2004). Except for *Candida dublinensis*, all *Candida* species were uniformly susceptible to fluconazole, voriconazole, amphotericin B and caspofugin. The multi-drug resistance displayed by *C. dublinensis* in this study remains the most worrisome. *C. dublinensis* isolates showing multidrug resistance have been reported, especially associated with oral candidiasis in HIV/AIDS patients (Quindós *et al.*, 2000). This further buttresses our notion that a significant portion of women in this study may have been HIV positive. *C. dublinensis* is very closely related to *C. albicans*. Antifungal resistance in both species has been linked to multidrug transporters encoded by *MDR1* and *CDR2* genes (Moran *et al.*, 1998) as well as point mutations in the *FUR1* and *FCA1* genes encoding enzymes that play a role in pyrimidine salvage pathway (Hope *et al.*, 2004).

Our study reports on the prevalence of candiduria among women visiting a tertiary hospital in Gaborone, Botswana. The high prevalence of antifungal resistance, especially among *Candida* species other than *C. albicans* is a cause of significant public health concern. It is suggested that vigilance be increased among healthcare workers in revising antifungal susceptibility testing and prescription regimen in order to curb antifungal resistance in Botswana due to *Candida* species.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Biological Sciences, University of Botswana for facilities and support.

REFERENCES

- Alenzi FQB (2016). Virulence factors of *Candida* species isolated from patients with urinary tract infection and obstructive uropathy. *Pakistan Journal of Medical Sciences* **32**(1) 143-146
- Anaissie EJ, McGinnis MR and Pfaller MA (2003). *Clinical Mycology* (1st edition), (USA, Philadelphia: Churchill Livingstone) 500.
- Baqai R, Aziz M and Rasool G (2008). Urinary tract infections in diabetic patients and biofilm formation of uropathogens. *Infectious Disease Journal of Pakistan* **17**(1) .21-24.
- Behzadi P, Behzadi E, Yazdanbod H, Aghapour R, Cheshmeh MA and Omran DS (2010). Urinary tract infections associated with *Candida albicans*. *Mædica Journal of Clinical Medicines* **5** 277-279.
- Birinci A, Cihan CC and Bilgin K (2006). Investigation of phospholipase activity of *Candida* species isolated from different clinical samples. *Microbiological Bulletin* **39** 205-209.
- Brito LR, Guimarães T, Nucci M, Rosas RC, Paula Almeida L, Da Matta DA and Colombo AL (2006). Clinical and microbiological aspects of candidemia due to *Candida parapsilosis* in Brazilian tertiary care hospitals. *Medical Mycology* **44**(3) 261-266.
- Chen YC, Eisner JD, Kattar MM, Rassoul-Barrett SL, LaFe K, Yarfitz SL, Limaye AP and De Backer MD, Ilyina T, Ma XJ, Vandoninck S, Luyten WH and Bossche HV (2001). Genomic profiling of the response of *Candida albicans* to itraconazole treatment using a DNA microarray. *Antimicrobial Agents and Chemotherapy* **45**(6) 1660-1670.
- Debora K, Simão L, Marines M and Jacyr P (2007). Comparison of agents and sensitivity profile in urinary tract infection in diabetic and non-diabetic patients. *Clinical Infectious Disease* **5** 363-367.
- Douglas LJ (2003). *Candida* biofilms and their role in infection. *Trends in Microbiology* **11**(1) 30-36.
- Dwyer PL and O'Reilly M (2002). Recurrent urinary tract infection in the female. *Current Opinion in Obstetrics and Gynecology* **14** 537-543.

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- El Sheikh SM and Johargi AK (2000).** Bacterial and Fungal infections among diabetics. *Journal of King Abdulaziz University-Medical Sciences* **8** 41-48.
- Ferrer C, Colom F, Frases S, Mulet E, Abad JL and Alió JL (2001).** Detection and identification of fungal pathogens by PCR and by ITS2 and 5.8 S ribosomal DNA typing in ocular infections. *Journal of Clinical Microbiology* **39**(8) 2873-2879.
- Foxman B (2002).** Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *American Journal of Medicines* **113**(1A) 5-13.
- Fujita SI, Senda Y, Nakaguchi S and Hashimoto T (2001).** Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *Journal of Clinical Microbiology* **39**(10) 3617-3622.
- Haider G, Zehra N, Munir AA and Haider A (2010).** Risk factors of urinary tract infection in pregnancy. (JPMA) *The Journal of the Pakistan Medical Association* **60**(3) 213-216.
- Hierro N, Gonzalez A, Mas A and Guillamón JM (2004).** New PCR-based methods for yeast identification. *Journal of Applied Microbiology* **97**(4) 792-801.
- Hope WW, Taberner L, Denning DW and Anderson MJ (2004).** Molecular mechanisms of primary resistance to flucytosine in *Candida albicans*. *Antimicrobial Agents and Chemotherapy* **48** 4377–4386.
- Ibadin MO, Onunu A and Ukoh G (2006).** Urinary tract infection in adolescents/young adult Nigerians with acquired human immunodeficiency disease in Benin City. *Journal of Biomedical Research* **5** 55–60.
- Kalpna S, Hegadi SS and Ramesh K (2015).** Characterization and antimicrobial susceptibility testing of uropathogens from urinary tract infections. *International Journal of Current Microbiology and Applied Sciences* **4**(2) 1010-1016.
- Manani TA, Collison EK and Mpuchane S (2006).** Microflora of minimally processed frozen vegetables sold in Gaborone, Botswana. *Journal of Food Protection* **69**(11) 2581-2586.
- Matsheka MI, Mpuchane S, Gashe BA, Allotey J, Khonga EB, Coetzee SH and Murindamombe G (2014).** Microbial quality assessment and predominant microorganism of biltong produced in butcheries in Gaborone, Botswana. *Food and Nutrition Sciences* **5**(17) 1668.-1678.
- Moran GP, Sanglard D, Donnelly SM, Shanley DB, Sullivan DJ and Coleman DC (1998).** Identification and expression of multidrug transporters responsible for fluconazole resistance in *Candida dubliniensis* *Antimicrobial Agents and Chemotherapy* **42**(7) 1819-1830.
- Naglik JR, Scott J and Rahman D (2005).** Serum and saliva antibodies do not inhibit *Candida albicans* SAP2 proteinase activity using a BSA hydrolysis assay. *Medical Mycology* **43** 73-77.
- Nimri L and Batchoun R (2010).** Community-acquired urinary tract infections in a rural area: Predominant uropathogens, and their antimicrobial resistance. *Webmed Central Microbiology* **1**(9) WMC006793.
- Orhue PO (2014).** Prevalence of uropathogenic bacterial isolates from urinary tract infections: A case study of university of Benin teaching hospital, Benin City, Nigeria. *International Journal of Microbiological Applications* **1**(2) 18-22.
- Payam B, Elham B, Hodjat Y, Raghiyyeh A and Djaafar SO (2010).** Urinary Tract Infections Associated with *Candida albicans*. *A Journal of Clinical Medicines* **5**(4) 277-279.
- Paz-Bailey G, Rahman M, Chen C, Ballard R, Moffat HJ, Kenyon T, Kilmarx PH, Totten PA, Astete S, Boily MC and Ryan C (2005).** Changes in the etiology of sexually transmitted diseases in Botswana between 1993 and 2002: implications for the clinical management of genital ulcer disease. *Clinical Infectious Diseases* **41**(9) 1304-1312.
- Pfaller MA and Diekema DJ (2004).** Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. *Clinical Microbiology and Infection* **10** 11-23.
- Preethishree P, Rai R, Kumar KV, Pai KA and Bhat UP (2016).** Uropathogens and their antibiotic susceptibility pattern at a tertiary care teaching hospital in Coastal Karnataka, India. *International Journal of Current Microbiological Applications* **5**(1) 23-31.

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Quindós G, Carrillo-Muñoz AJ, Arévalo MP, Salgado J, Alonso-Vargas RIO, Rodrigo JEM, Ruesga MIAT, Valverde A, Pemán J, Cantón E and Martín-Mazuelos E (2000). *In vitro* susceptibility of *Candida dubliniensis* to current and new antifungal agents. *Chemotherapy* **46**(6) 395-401.

Ramage G, Bachmann S, Patterson TF, Wickes BL and López-Ribot JL (2002). Investigation of multidrug efflux pumps in relation to fluconazole resistance in *Candida albicans* biofilms. *Journal of Antimicrobial Chemotherapy* **49**(6) 973-980.

Ramesh H and Agarwal KK (2012). Urinary Tract Infections in Women. *Indian Journal of Clinical Practice* **23**(4) 187-192.

Shah LJ, Vaghela GM and Mahida H (2015). Urinary tract infection: Bacteriological profile and its antibiotic susceptibility in western India. *National Journal of Medical Research* **5**(1) 71-74.

Sobel JD, Chaim W, Nagappan V and Leaman D (2003). Treatment of vaginitis caused by *Candida glabrata*: use of topical boric acid and flucytosine. *American Journal of Obstetrics and Gynecology* **189**(5) 1297-1300.

Stamm WE (2001). An epidemic of urinary tract infections? *Northern English Journal of Medicines* **345** 1055-1057.

Statistics Botswana (2014). BAIS IV Final Results Presentation. *Stats Brief: Preliminary Results, Botswana AIDS Impact Survey IV (BAIS IV), 2013*. (Government of Botswana, Gaborone, Botswana).

Tortorano AM, Kibbler C, Peman J, Bernhardt H, Klingspor L and Grillot R (2006). Candidaemia in Europe: epidemiology and resistance. *International Journal of Antimicrobial Agents* **27**(5) 359-366.

White TJ, Bruns T, Lee S and Taylor J (1990). Amplification and direct sequencing of fungi ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (edition), *PCR Protocols: A Guide to Methods and Applications*, (Academic Press, San Diego, USA) 315–322.

Yesudhasan BL and Mohanra MK (2015). *Candida tropicalis* as a predominate isolates from clinical specimens and its antifungal susceptibility pattern in a tertiary hospital in Southern India. *Journal of Clinical and Diagnostic Research* **9**(7) 14 – 16.