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Urinary tract candidiasis in HIV+ patients and sensitivity patterns of recovered *Candida* species to antifungal drugs in Dschang District Hospital (Cameroon)

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ABSTRACT

The incidence of *Candida* urinary tract infections is gradually on the rise and is an important public health problem. The aim of our study was to determine the prevalence of urinary tract candidiasis (candiduria) in HIV positive patients in Dschang District Hospital and the antifungal susceptibility test of isolates. A total of 285 patients were recruited for this study. Midstream urine samples were collected and processed using standard mycological techniques. *Candida* isolates were identified base on the colony color on CHROMagar. Antifungal susceptibility testing of the isolates was performed by the broth dilution method using four commonly used antifungals. Results showed that 22% of patients had *Candida* spp in their urine. *Candida albicans* had a proportion of 37% against 63% for non-*albicans Candida*. Of the 53 isolates tested, ketoconazole had the highest percentage of resistance (88.6%) follow by fluconazole (64.1.6%), amphotericin B (56.6%) and nystatin (49.0%). The highest sensitivity was observed with nystatin (33.9%) while the lowest was found with ketoconazole (5.6%). In conclusion, the prevalence of candiduria among HIV positive patients in this study was 22%. *Candida albicans* remain the most frequently involved *Candida* species. Azole antifungals showed the highest resistance rate against all the *Candida* species isolates.
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Keywords: HIV+ patients, Candiduria, *Candida* spp, Antifungal susceptibility.

INTRODUCTION

Candidiasis is a common opportunistic infection in HIV-infected patients (Kauffman, 2014). The spectrum of *Candida* infection is diverse, starting from asymptomatic colonization to pathogenic forms. The low absolute CD4+ T-lymphocyte count has traditionally been cited as the greatest risk factor for the development of oropharyngeal candidiasis and current guidelines suggest increased risk once CD4+ T lymphocyte counts fall below 200 cells/µL

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(Anwar et al., 2012). Candiduria presents as an increasingly common nosocomial infection, which may involves urinary tract. Spectrum of disease is varying from asymptomatic candiduria to clinical sepsis. Several reports showed that the frequency of urinary tract infection due to yeasts has increased during the last decade (Yashavanth et al., 2013). Changes in the clinical severity of candidiasis and the *Candida* species prevalence profile may be a reflection of immunological changes in HIV positive patients. Though *Candida albicans* is the most frequently isolated species as a colonizer and pathogen of mucosa, other *Candida* species, such as *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. dubliniensis*, *C. guilliermondii*, *C. parapsilosis*, *C. kefyr*, and *C. pelliculosa*, have become a significant cause of infection in patients with HIV infection (Fusi et al., 2011; Kaur et al., 2016). The clinical importance of these non-*albicans* *Candida* species lies in the fact that they are usually less susceptible to the more commonly used azole antifungal drugs, a factor that poses significant difficulties in effective treatment (Maheshwari et al., 2016). During the last few decades, the spectrum of infections has undergone a drastic change; organisms with minimal or no pathogenic role have emerged as potent pathogens and organisms once susceptible have become multidrug resistant (Ojieabu et al., 2012; Seifi et al., 2013). Emergence of non-*albicans* *Candida* species as a cause of refractory candidiasis, particularly in patients with advanced immunosuppression and problem of resistance to azoles and other antifungal agents in the *Candida* species is a point of concern (Lozes et al., 2012; Deorukhkar et al., 2014). Therefore, this variability in the behaviour of different *Candida* spp. and the increasing number of clinical isolates resistant to current antifungal therapies highlight the need for antifungal susceptibility testing to monitor the antifungal resistance of these microorganisms. The modern mycologist has an important role to play in several aspects relating to these organisms, including detection, identification, epidemiological analysis, and therapy in an attempt to better understand these pathogens and provide an effective cure (Maheshwari et al., 2016). This could guide the therapeutic choice and the clinical treatment (Terças et al., 2017). The overall prevalence of urinary candidiasis among HIV infected patients was found to be 18.8% and 36.2% in two localities of Cameroon, Buea and Yaounde respectively (Lohoué et al., 2005; Longdoh et al., 2013). However, in the west region of Cameroon, candiduria in HIV infected patients is not looking for in general practice. Yet it can be the source of disseminated infection in immunosuppressed individuals. The aim of the present study was to determine the frequency of candiduria in HIV positive patients in Dschang District hospital and to determine the susceptibility to antifungal drugs of *Candida* species isolated in the view to contributing to a better management of *Candida* infections in HIV+ patients.

MATERIALS AND METHODS

Study population

This study was a cross-sectional study carried out during a period of 9 months, from June 2013 to February 2014 at the Dschang District Hospital. After the aim of research was clearly explained to the patients who fulfill the included criteria, patients gave written consent. The study was conducted in accordance with the declaration of Helsinki, and the protocol was approved by the Cameroon Bioethics Initiative Ethics Review and Consultancy Committee (CAMBIN

ERRC) and an ethics clearance with reference number CBI/297/ERCC/CAMBIN was issued.

Sample collection and culture

The urine samples were collected from patients and were processed for yeasts at the laboratory of Microbiology and Antimicrobial substances of the Biochemistry Department, University of Dschang. None of the patients used antifungal during the sampling. After cleaning the genital region with a dakin solution (antiseptic), midstream urine were collected from patients into sterile urine bottles in the morning and maintained at 4 °C during transportation to the laboratory for analysis. 100 µl of each uncentrifuged urine sample was cultured on CHROMagar *Candida* (CHROMagar *Candida*®) containing chloramphenicol plates as lawn and incubated at 25 °C for 48 h. Yeasts were identified to the species level by standard methods: microscopic morphology, characteristic color displayed on CHROMagar.

Antifungal susceptibility testing

The sensitivity of obtained isolates was tested vis-a-vis of four antifungal namely: fluconazole, nystatin, amphotericin B and ketoconazole. The minimum inhibitory concentration (MIC) of the antifungal were determined by liquid medium microdilution technique according to the protocol described by the National Committee and Clinical Laboratory Standard (NCCLS, 2002). The interpretative breakpoints were the following: Fluconazole: Susceptible if MIC < 8 µg/mL; Intermediary if MIC was between 16 to 32 µg/mL; Resistant if MIC > 64 µg/mL. Ketoconazole: Susceptible if MIC < 0.125 µg/mL; Intermediary if MIC was between 0.25 to 0.5 µg/mL; Resistant if MIC > 1 µg/mL. Amphotericin B and nystatin: Susceptible if MIC < 1 µg/mL; Intermediary if MIC was between 2 to 4 µg/mL; Resistant if MIC > 4 µg/mL (Thérèse et al., 2006)

RESULTS

Our study population was made up of 285 patients of which 224 women (79%) and 61 men (21%) with a sex ratio of 3.7 in favor of the women. The average of global age of patients was 39.69 ± 11 years with extremes of 4 years (minimum) and 69 years (maximum). In this population, 4% (11) were children (0-20), 78% (223) were young adults (21-49 years) and 18% (21) of more than 50 years (Table 1).

Five different *Candida* species were identified namely *C. albicans, C. glabrata*, *C. krusei C. dubliniensis* and *C. tropicalis* (Figure 1). The most prevalent species was *C. albicans* 37.5% followed by *C. glabrata* 19.4%, *C. krusei,* 16.6%, *C. tropicalis* 11.1% and *C. dubliniensis* 8.3%. There was 6.9% of non-identified species. It is noteworthy that non-*albicans Candida* species emerged with 55.4% against 37.5% for *Candida albicans* species (Figure 1).

Among the 224 women, 21% were positive to candiduria while 6% of 64 men were positive to candiduria. Therefore, women were more affected by candiduria than men. Regarding the age groups, 21 to 49 range was the most affected 22% (48/223), follow by the ≥50 age in which 19% of person were affected (4/11). About CD4+ level, 42% had 201-500 count/ mm^3 followed by patients with CD4+ ≤200 (31%) (Table 2). There was an unequal distribution of the *Candida* species in the two sexes, with a predominance of all the species among women, except *Candida dubliniensis* which was predominant in men. The age range of 21-49 years was the age groups where all *Candida* species were the most represented, particularly *Candida*

dubliniensis followed by *Candida albicans* and *Candida glabrata*. Patients with 201-500 counts/mm^3 CD4+ level were the most affected by candiduria (Table 2).

The results of the antifungal susceptibility test showed a wide range of MIC value varying from <0.125 µg/mL to >256 µg/mL with the smallest geometric mean of <0.54 µg/mL. Regarding the range of inhibitory concentration and geometric mean, nystatin had the smallest range and geometric mean values for all isolates follow by amphotericin B (Table 3). All the *Candida albicans* species were resistant to nystatin and ketoconazole. Ketoconazole had the highest percentage of resistance towards *Candida* spp isolates (88.6%), follow by fluconazole (64.1.6%), amphotericin B (56.6%) and nystatin (49.0%). The highest sensitivity was observed with nystatin (33.9%) while the lowest was found with ketoconazole (5.6%) (Table 4).

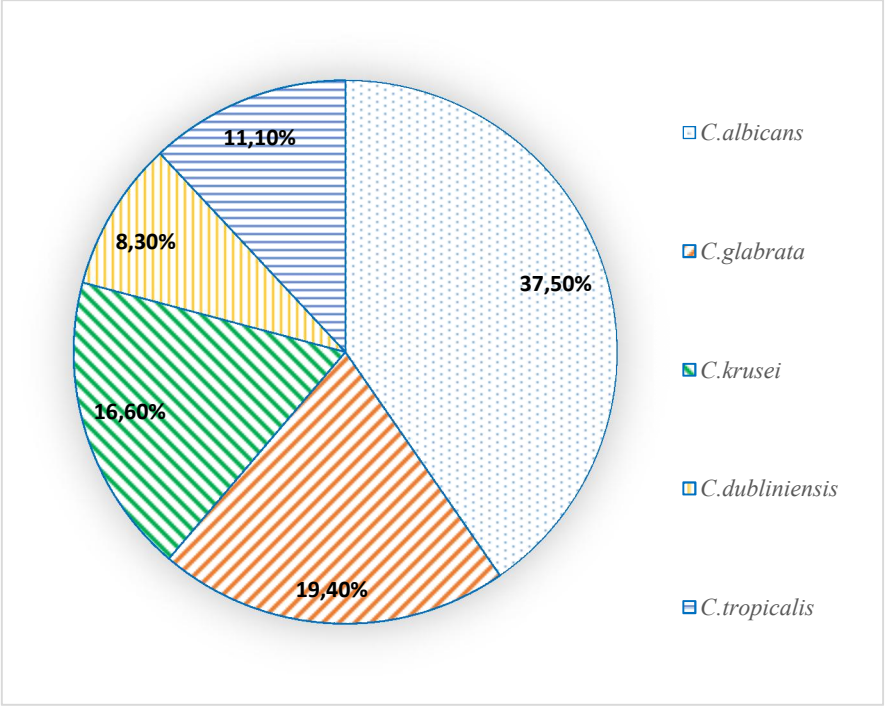

Figure 1: Distribution of identified *Candida* species.

Table 1: Distribution of candiduria according to sex, groups of age and CD4+ level.

Sex		**Age groups (year)**			**CD4+ level (/mm^3)**		
Female	Male	≤20	21-49	≥50	≤200	201-500	>500
47 (21%)	6 (10%)	0 (0%)	48 (22%)	4 (19%)	16 (31%)	22 (42%)	4 (8%)

Table 2: Distribution of different *Candida* species according to sex, age groups and CD4+ level.

		Ca	***Cg***	***Ck***	***Ct***	***Cd***	**Total**
Sex	Female	17 (96%)	11 (93%)	10 (83%)	6 (86%)	3 (83%)	47 (88.6%)
	Male	1 (4 %)	1 (7%)	2 (17%)	1 (14%)	1 (17%)	6 (11.30%)
Age group (year)	≤ 20	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	21-49	16 (93%)	11 (93%)	11 (92%)	6 (87%)	4 (100%)	48 (90.5%)
	≥ 50	2 (7%)	1 (7%)	1 (8%)	1 (23%)	0 (0%)	4 (7.5%)
CD4+ level (/mm^3)	≤ 200	14 (77 %)	11 (93%)	10 (83%)	6 (87%)	3 (83%)	22 (41.5%)
	201-500	3 (17%)	0 (0%)	2 (17%)	1 (13%)	1 (17%)	16 (30.1%)
	> 500	1 (6%)	1 (7%)	0 (0%)	0 (0%)	(0 0%)	4 (7.5%)

Ca: Candida albicans, *Ck: Candida krusei, Cg: Candida glabrata, Candida tropicalis, Cd: Candida dubliniensis*

Table 3: Minimum inhibitory concentration (MICs) values of candida isolated to ketoconazole, Amphotericin B, fluconazole and nystatin.

		MIC (µg/mL)				
Antifungals		***Ca* (n=18)**	***Ck* (n=12)**	***Cg* (n=12)**	***Ct* (n=7)**	***Cd*(n=4)**
Ketoconazole	Range	4->256	<0.125->256	<0.125->256	2-256	0.125-256
	GM	48.87	>5.65	7.12	32	5.65
Amphotericin B	Range	0.25-128	2->256	1->256	0.25-128	1-4
	GM	2.61	16	>13.92	5.94	1.41
Fluconazole	Range	8->256	0.5->256	1->256	8->256	2->256
	GM	>155.17	>42.71	25.39	>128	26.90
Nystatin	Range	<0.125-8	0.125->256	<0.125-8	0.125-4	1-64
	GM	<0.54	>1.41	1.18	<0.82	2.82

GM: Geometric mean, *Ca: Candida albicans*, *Ck: Candida krusei, Cg: Candida glabrata, Candida tropicalis, Cd: Candida dubliniensis.* n = number of isolate tested.

Table 4: Susceptibility patterns of *Candida* isolates from ketoconazole, Amphotericin B, fluconazole and nystatin.

Antifungals		***Ca* (n=18)**	***Ck* (n=12)**	***Cg* (n=12)**	***Ct* (n=7)**	***Cd* (n=4)**	**Total**
Ketoconazole	S	0 (0 %)	1 (8.3%)	1 (8.3%)	0 (0%)	1 (25%)	3 (5.6%)
	I	0 (0 %)	1 (8.3%)	2 (16.7%)	0 (0%)	0 (0%)	3 (5.6%)
	R	18 (100 %)	10(83.3%)	9 (75%)	7 (100%)	3 (75%)	47 (88.6%)
Amphotericin B	S	6 (33.3%)	1 (8.3%)	1 (8.7%)	3 (42.9%)	3 (75%)	14 (26.4%)
	I	3 (16.6%)	3 (25 %)	2 (16.7%)	0 (0%)	1 (25%)	9 (34.6%)
	R	9 (50%)	8 (66.7%)	9 (75%)	4 (57.1%)	0 (0%)	30 (56.6%)
Fluconazole	S	0 (0%)	2 (16.7%)	4 (33.3%)	1 (14.2%)	1 (25%)	8 (15.0%)
	I	1(12.5%)	4 (33.3%)	4 (33.3%)	0 (0%)	2 (50%)	11 (20.7%)
	R	17(87.5 %)	6 (50%)	4 (33.3%)	6 (85.7%)	1 (25%)	34 (64.1%)
Nystatin	S	0 (0%)	8 (66.7%)	7 (58.3%)	0 (0%)	3 (75%)	18 (33.9%)
	I	0 (0%)	2 (16.7%)	4 (33.3%)	1 (20%)	0 (0%)	7 (13.2%)
	R	18 (100%)	2 (16.7%)	1 (8.3%)	4 (80%)	1 (25%)	26 (49.0%)

Ca: Candida albicans, *Ck: Candida krusei, Cg: Candida glabrata, Candida tropicalis, Cd: Candida dubliniensis.*
n = number of isolate tested, R: resistant, I: Intermediary, S: sensitive.

DISCUSSION

Candida infections are the most common fungal infections in HIV patients and *Candida albicans* is the most involved (Guessous-Idrissi et al., 2007). However, there is more implication of non-*albicans Candida* in fungal infection (Fleming et al., 2002). The results of the prevalence of etiologic agents of candiduria in this study showed that non-*albicans Candida* represented 55.7% against 37.5% for *Candida albicans* species, confirming the tendency of the general increase prevalence of non-*albicans Candida* in candidiasis (Abi-Said et al., 1997; Fleming et al., 2002). This study included 224 women (79%) and 61 men (21%) giving a sex ratio of 3.7 in favor of the women. Women are more exposed to infections than men, especially through their surface genital area and greater exposure. Esebelahie et al. (2013) previously reported the female gender as a significant risk factor for acquiring *Candida* infection in asymptomatic HIV patients attending a tertiary hospital in Benin City in Nigeria. In addition, the hormonal differences that exist between, the levels of estrogen and progesterone that are reduced in men could also explain this result. Indeed, *Candida albicans* has membrane receptors that bind progesterone colonization (Zlotnik et al., 2011). The distribution of HIV infected patients by age reported a mean overall age of 39.69 ± 11 years with a predominance of young adults. We also noticed a high prevalence of candiduria (92%) among young adults. These results corroborate with those of Lohoué et al. (1991) who reported the age group of 26 to 45 as being the most affected by the candiduria. Indeed, the HIV / AIDS pandemic, as well as candiduria would be the prerogative of young sexually active populations. According to the CD4+ cell level, high prevalence of candiduria is associated with low CD4+ cell counts (16% and 22% for CD4+ 200 / mm^3 and between 201 and 500 / mm^3 respectively, compared with 4% for >500 / mm^3). These findings are similar observation previously made by Longdoh et al. (2013) who reported that urinary candidiasis was more frequent in individuals with CD4+ T cell count below 200 cells/µL.

Treatment against candidiasis varies substantially depending on the anatomical localization of the infection, the immune status of the patient, and the isolated species (Rex et al., 2000). In order to understand the therapeutic differences in the isolates involved in candiduria study population, we tested the sensitivity of these isolates. Our findings indicated that *Candida albicans* isolates were highly resistant to ketoconazole and nystatin. Among non-*albicans Candida* species high resistance was observed with ketoconazole against *Candida tropicalis*. These results can be compared with those of Sangeorzan et al. (1994) which showed failure of azoles, more particularly fluconazole in the treatment of oropharyngeal candidiasis. On the other hand, Anane et al. (2006) reported a very high sensitivity to ketoconazole from *Candida albicans* isolates isolated from HIV-positive recruited from a district hospital in Douala-Cameroon. These results suggest the importance of antifungal susceptibility test before any prescription of antifungals; the inappropriated prescriptions could be responsible for resistant strains. It has been shown that, *Candida albicans* and non-*albicans Candida* species differ from one to another in their epidemiology, virulence and susceptibility to antifungals, verification of

specific implication of each species of *Candida* is required before any therapeutic decision (Rex et al., 2000).

Conclusion

The present study reiterates the prevalence of candiduria species among HIV positive patients in Dschang District hospital and their antifungal susceptibility pattern of *Candida* spp involved. The prevalence of candidiasis among HIV patients in this study was 22%. *Candida albicans* remain the most frequently involved *Candida* species. Azole antifungals showed the highest resistance rate against all the *Candida* species isolates. Therefore, the species identification of *Candida* isolates along with their antifungal susceptibility pattern can help the clinicians in better treating the patients with candiduria.

COMPETING INTERESTS

The authors declare that they have no competing of interests.

AUTHORS' CONTRIBUTIONS

ISY, FAK and JPD conceived and designed the experiments; AND, JD and CN performed the experiments; ISY, CN and J.D. analyzed the data; AND and CN drafted the manuscript and JPD finalized the paper.

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REFERENCES

Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. 1997. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin. Infect. Dis*., **24**(6): 1122-1128. DOI: http://www.jstor.org/stable/4460015.

Anane S, Khalfallah F. 2006. Diagnostic biologique des candidoses systémiques: difficultés et perspectives. *Pathol. Biol*., **55**(5): 262-272. DOI: 10.1016/j.patbio.2006.03.003.

Anwar KP, Malik A, Subhan KH. 2012. Profile of candidiasis in HIV infected patients. *Iran J. Microbiol.,* **4**(4): 204-209. DOI: http://ijm.tums.ac.ir/index.php/ijm/article/view/669/0.

Deorukhkar SC, Saini S, Mathew S. 2014. Non-*albicans Candida* Infection: An emerging threat. *Interdiscip. Perspect. Infect. Dis*., **2014**: 615958. DOI: 10.1155/2014/615958.

Esebelahie NO1, Enweani IB, Omoregie R. 2013. *Candida* colonisation in asymptomatic HIV patients attending a tertiary hospital in Benin City, Nigeria. *Libyan J. Med*., **8**: 20322. DOI: 10.3402/ljm.v8i0.20322.

Fleming RV, Walsh TJ, Anaissie EJ. 2002. Emerging and less common fungal pathogens. *Infect Dis Clin North Am*., **16** (4): 915-933. DOI: http://dx.doi.org/10.1016/S0891-5520 (02)00041-7.

Fusi NCNK, Payne VK, Asakizi AN. 2012. Non-classical reproductive tract infections on the rise in women in Dschang, Cameroon. *Int. J. Biol. Chem. Sci.,* **6**(5): 2016-2025. DOI: http://dx.doi.org/10.4314/ijbcs.v6i5.11

Guessous-Idrissi N, Essari A, Abdallaoui S, Youssouf M. 2007. Première identification de *Candida dubliniensis* au centre hospitalier universitaire Ibn Rochd de Casablanca, Maroc. *J Mycol Med*., **17** (2): 77-81. DOI: 10.1016/j.mycmed.2007.03.003.

Kauffman CA. 2014. Diagnosis and management of fungal urinary tract infection. *Infect. Dis. Clin. North. Am.*, **28**(1): 61-74. DOI: 10.1016/ j.idc.2013.09.004.

Kaur R, Dhakad MS, Goyal R, Kumar R. 2016. Emergence of non-*albicans Candida* species and antifungal resistance in intensive care unit patients. *Indian. J. Microbiol. Res*., **3**(4): 398-400. DOI: https://doi.org/10.1016/j.apjtb.2015.12.019

Lohoué J, Nomo O A, Same E A. (1991). Candidose et SIDA à Yaoundé. *Bull Soc Pathol Exot*., **84**(2): 133-135.

Lohoué JP, Angwafo III FF, Kechia FA, Noukeu ND. 2005. Candiduria in HIV Infected Patients in Yaoundé, Cameroon. *Afr. J. Urol*., **11**(1): 61-65.

Longdoh A. Njunda, Jules C. N. Assob, Shey D. Nsagha, Henri L. F. Kamga, Ejong C. Ndellejong, Tebit E. Kwenti. 2013. Oral and urinary colonisation of *Candida* species in HIV/AIDS patients in Cameroon. *Basic Sci. Med.*, **2**(1):1-8. DOI: 10.5923/j.medicine.20130201.01.

Lozes E, Ahoussinou C, Agassounon M, TchibozoDjikpo, Dahouegnon E, Ahossouhe N, Acoty A, De Souza C. 2012. Variabilité du taux des lymphocytes CD4 et de la charge virale chez les personnes vivant avec le VIH sous thérapie antirétrovirale : cas de l'hôpital saint jean De Dieu de Tanguieta (Benin). *Int. J. Biol. Chem. Sci.,* **6**(2): 650-656. DOI: http://dx.doi.org/10.4314/ijbcs.v6i2.9.

Maheshwari M, Kaur R, Chadha S. 2016. *Candida* species prevalence profile in HIV seropositive patients from a major tertiary care hospital in New Delhi, India. *J Pathog*., **2016**: 6204804. DOI: 10.1155/2016/6204804.

National Commitee for Clinical Laboratory Standards (NCCLS). 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard (NCCLS document M27-A2) Villanova, PA: National Committee for Clinical Laboratory Standards.

Ojieabu WA, Femi-Oyewo MN, Ojieabu CI. 2012. Impact of educational status on HIV/AIDS knowledge, attitude and misconception among pregnant women. *Int. J. Biol. Chem. Sci.,* **6**(4): 1582-1592. DOI: http://dx.doi.org/10.4314/ ijbcs.v6i4.18.

Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, Dismukes WE, Edwards JE. 2000. Practice guidelines for the treatment of candidiasis. *Clin. Infect. Dis*., **30** (4): 662-678. DOI: 10.1086/313749.

Sangeorzan JA, Bradley SF, He X, Zarins L T, Ridenour GL, Tiballi RN, Kauffman CA. 1994. Epidemiology of oral candidiasis in HIV-infected patients: colonization, infection, treatment, and emergence of fluconazole resistance. *Am J Med*., **97**(4): 339-346. DOI: 10.1016/0002-9343(94)90300-X.

Seifi Z, Azish M, Salehi Z, Mahmoudabadi AZ, Shamsizadeh A. 2013. Candiduria in children and susceptibility patterns of recovered *Candida* species to antifungal drugs in Ahvaz. *J. Nephropathology*, **2**(2): 122-128. DOI:10.12860/JNP.2013.20.

Terças AL, Marques SG, Moffa EB, Alves MB, de Azevedo CM, Siqueira WL, Monteiro CA. 2017. Antifungal Drug Susceptibility of *Candida* Species Isolated from HIV-Positive Patients Recruited at a Public Hospital in São Luís, Maranhão, Brazil. *Front. Microbiol*., **8**: 298. DOI: 10.3389/fmicb.2017.00298.

Thérèse L., Bagyalakshmi R., Madhavan N., Deepa P. 2006. *In vitro* suscepbility testing by agar dillution method to determine the minimum inhibitory concentrations of amphotericin B, fluconazole and ketoconazole agains ocular fungal isolates. Indian *J. Med. Microbiol*., **24**(4): 273-279. DOI: 10.4103/0255-0857.29386.

Yashavanth R, Shiju MP, Bhaskar UA, Ronald R, Anita KB. 2013. Candiduria: prevalence and trends in antifungal susceptibility in a tertiary care hospital of mangalore. *J. Clin. Diagn. Res*., **7**(11): 2459-2461. DOI: 10.7860/JCDR/2013/6298.3578.

Zlotnik A, Ohayon S, Gruenbaum BF, Gruenbaum SE, Mohar B, Boyko M, Klin Y, Sheiner E, Shaked G, Shapira Y, Teichberg. 2011. Determination of factors affecting glutamate concentrations in the whole blood of healthy human volunteers. *J Neurosurg Anesthesiol.,* **23**(1): 45-49. DOI: 10.1097/ANA.0b013e3181f82a8f.