African Journal of Integrated Health Vol 7: N°1; June 2017 P. 38-42

RESEARCH ARTICLE

Prevalence of Candiduria in Diabetic Patients attending the Bafoussam Regional Hospital (West Cameroon) and Antifungal Susceptibility of the Isolates

Ngueguim D Aude[1], Iwewe Somo Y[2], Kechia F. Agem[2,3], Nangwat C[1], Diesse JM[1], Tchuenguem Tchuente R[1], Dzoyem JP[1,*]

[1]Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon.
[2]Medical/Clinical Mycology Laboratory, Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Cameroon.
[3]Department of Biomedical Sciences, Faculty of Health Sciences, University of Bamenda, Cameroon.

*** Corresponding author**: jpdzoyem@yahoo.fr; Tel.: +237-676091031

Objective: the aim of this study was to collect epidemiological and biological data on the etiologic agents of candiduria in diabetics' patients attending Bafoussam Regional Hospital in Cameroon.

Methods: *Candida* species were identified based on colony morphology on chromagar Candida. The broth microdilution method was subsequently used to evaluate the sensitivity of the isolates towards five commonly used antifungals.

Results: of the 120 samples collected, 40 (33.3%) cultures were positive for candida species, of which colony counts were >104 cfu/ml in 24 (20%) of the cases. Incidence rate of candida spp. was determined as *C. albicans* 19 (31.6%), *C. krusei* 14 (23.3%), *C. glabrata* 12 (20%), *C. parapsilosis* 10 (16.7%), *C. tropicalis* 3 (5%) and *C. dubliniensis* 2 (3.3%). Minimum inhibitory concentration values ranged from 0.0312 µg/ml to >256 µg/ml. Most of the *Candida* spp were resistant against amphotericin b and nystatin with 81.7% and 53.3% of resistance respectively. The majority of candida isolates were sensitive to ketoconazole (43.3%), follow by fluconazole (40.0%), nystatin (21.7%), and amphotericin b (5.0%). None of the *Candida* species identified was sensitive to terbinafine.

Conclusion: the prevalence of candiduria in the population studied was 33.3%. *C. albicans* as well as non-albicans species being involved. All the *Candida* species identified presented high level of resistance towards amphotericin B. Therefore, it is important to search routinely for yeast in the urine of diabetic patients to detect candidiasis, and to perform antifungal susceptibility tests to *Candida* isolates in order to establish antifungal therapy for these patients.

Keywords: Candiduria, Diabetes, Antifungal Susceptibility

INTRODUCTION

Rarely observed in the general population, candiduria is the presence of *Candida* species in the urine [1]. It is increasingly becoming an important subgroup of nosocomial urinary tract infections (10-15%) and almost all are caused by *Candida* spp [2]. Moreover, candiduria is a marker for hematogenous seeding in the kidney [3]. It may indicate bladder colonization due to indwelling catheters and primary or disseminated candidiasis [4]. Amongst the risk factors of candiduria is diabetes. Several studies demonstrated that diabetes may also predispose patients to more severe infections of the upper urinary tract, this being achieved in nearly 80% of urinary tract infections in diabetics' patients [5,6,7]. All *Candida* species are capable of causing UTIs, and in many centers worldwide, non-*albicans* (NA) species now predominate. Among

Candida species, *C. albicans* is the most common isolated species according to epidemiological studies of fungal UTI [8]. Although *Candida albicans* is the major cause of candiduria, non-*albicans Candida* (NAC) have emerged as important opportunistic pathogens. The NAC spp. are not only well adapted to the urinary tract but also are difficult to eradicate than *C. albicans*. These newly emerging non-*albicans*, including *C. glabrata*, *C. krusei*, and *C. parapsilosis* are implicated as causative agents. However, these strains show more resistance to antifungal drugs, especially to the first-line treatments. The development of resistance to antifungal drugs has become increasingly important and there is growing evidence of shifts to more resistant strains [9]. Some previous studies showed an increase in the incidence of *C. glabrata* infection which might be due to the extensive and long-term utilization of antifungal drugs such as azoles. Thus, the differentiation of diverse species of *Candida* in the laboratory seems imperative. In Cameroon candiduria in diabetic patients is a routine in general practice. Yet it can be the source of disseminated infection in immunosuppressed individuals. Therefore, this study was done with the aim of isolation and identification of different *Candida* species involved in candiduria in diabetic patients in Bafoussam regional hospital (Cameroon) and to determine the susceptibility to five commonly used antifungals including amphotericin B, ketoconazole, fluconazole, terbinafine and nystatin to demonstrate the local resistance profiles and to guide empirical treatment for clinicians.

MATERIALS AND METHODS

This study was a cross-sectional study carried out during a period of July to October 2015 at the Bafoussam Regional Hospital. All subjects gave their informed consent for inclusion before they participated in the study. An authorization to carry out the study in the hospital was obtained with reference number 494/L/MINSANTE/SG/DSPO/HRB/D. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the "*Comité National d'Ethique de la Recherche pour la Santé Humaine du Centre*" (CRERSHC) and an ethical clearance with reference number CE N° 000645-1/CRERSHC/2016 was issued. Our study population was comprised of diabetes patients of all ages and of both sexes, who came for their follow up or treatment monitoring and presenting or not, symptoms of urinary tract infection. Patients who were under antifungal therapy were excluded from this study.

After cleaning vulvo-region with Dakin"s solution, mid-stream urine were collected from patients into sterile urine bottles in the morning and maintained at 4^0C during transportation to the laboratory for analysis. The CHROMagar *Candida* (Media Mage, Johannesburg, South Africa) culture medium used for the culture of microorganisms was prepared as directed by the manufacturer and poured into petri dishes. It was then allowed to solidify and later inoculated with 50µL of each un-centrifuged urine sample and incubated at 37°C for 48h. The number of colonies on each plate were counted and recorded based on colony colours. Since discrepancies in CFU criteria to diagnose candiduria have not yet been adequately addressed, the following CFU cut-off were considered in this study: a quantitative culture with a colony count of $\geq 10^4$ CFU/mL of urine were associated with infection while a colony count of $<10^4$ CFU/mL of urine was associated with colonization [5,10].

The sensitivity of obtained isolates was tested vis-a-vis of five antifungal namely: fluconazole (FLC), nystatin (NYS), amphotericin B (AMB), ketoconazole (KTC) and terbinafine (TBF). The MICs were determined by liquid medium microdilution technique according to the protocol described by the National Committee and Clinical Laboratory Standard (NCCLS) [11].

Table 1, shows the interpretive criteria for the used antifungal drugs used including fluconazole, nystatin, amphotericin B, ketoconazole and terbinafine.

RESULTS

Table 1: Standard threshold values for MIC (µg/mL) interpretation.

Antifungals	Sensitive	Intermediate	Resistant
Ketoconazole	≤ 0,125	0,25-0,5	≥ 1
Amphotericin B	≤ 1		> 4
Fluconazole	≤ 8	16-32	≥ 64
Nystatin	≤ 1	2-4	> 4
Terbinafine	≤0,03	0,125-8	≥16

The study population consisted of 120 diabetics' patients including 67 females (55.8%) and 53 males (44.2%). The results of mycological analysis revealed that 40 samples yielded *Candida* species giving a total prevalence of 33.3% of candiduria in our population. Among the positive samples, 10 (25%) were males and 30 (75%) were females. *Candida* colonization ($<10^4$ CFU/mL) was seen in 36 (30%) patients while

infection (≥10^4 CFU/mL) was seen in 24 (20%) of the patients (Table 2).

Table 2: *Candida* species distribution by CFU/ml.

***Candida* species**	**Colonization (<10^4 CFU/mL)**		**Infection (≥10^4 CFU/mL)**		**Total**
	N	%	N	%	
C. albicans	14	23.3	5	8.3	19
C. krusei	8	13.3	6	10	14
C. glabrata	6	10	6	10	12
C. parapsilosis	3	5	7	11.7	10
C. tropicalis	3	5	0	0	3
C. dublinensis	2	3.3	0	0	2
Total	**36**	**60**	**24**	**40**	**60**

The distribution of all the *Candida* species isolates identified is shown in Figure 1. The most common species isolated was *C. albicans* 31.6%, followed by *C. krusei* (23.3%), *C. glabrata* (20%), *C. parapsilosis* (16.7%), *C. tropicalis* (5%), *and C. dubliniensis* (3.3%).

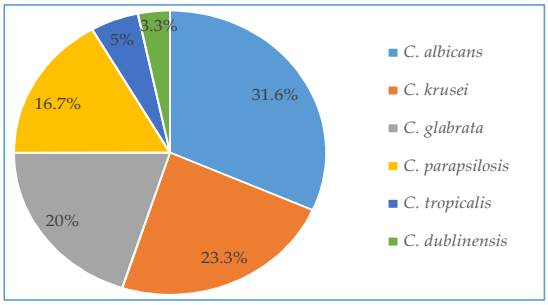

Figure 1: Distribution of *Candida* species isolates

Minimum inhibitory concentration (MICs) and geometric mean values of *Candida* isolates to antifungals are presented in Table 3.

MIC values ranged from 0.0312 µg/mL to >256 µg/mL. All the antifungal tested gave the lowest MIC value against at least one of the *Candida* species identified. Geometric mean values varied from 0.5 to >157.58 µg/mL. The antifungal susceptibility patterns of *Candida* isolates (Table 4) showed that most of the *Candida* spp were resistant against AMB and NYS with 81.7% and 53.3% of resistance respectively. The majority of *Candida* isolates were sensitive to KTC (43.3%), follow by FLC (40.0%), NYS (21.7%), and AMB (5.0%).

DISCUSSIONS

Urinary tract infections (UTIs) due to *Candida* species have become common in diabetic patients [12]. The aim of this study was the isolation and identification of different *Candida* species involved in candiduria in diabetic patients. The prevalence of candiduria in the study population was 33.3% among which 25% of females and 8.3% of males, indicating a female preponderance, with an overall male:female ratio being 1:3, suggesting that female sex is a risk factor for developing candiduria. Since colonization of vulvo vestibular area with *Candida* spp. is frequent in females, they are more at risk of developing candiduria due to ascending infection [2]. Significant candiduria was previously reported to be strongly associated with being female [3]. In this study, a total of 40 out of 120 urine samples showed the growth of *Candida* species. Among them non-*albicans Candida* species 41 (68.4 %), were predominant compared to *C. albicans* 19 (31.6%). Non-*albicans Candida* species included *C. krusei* (23.3%), *C. glabrata* (20%), *C. parapsilosis* (16.7%), *C. tropicalis* (5%), *C. dubliniensis* (3.3%). The species identification of *Candida* is important, as non-*albicans Candida* species are increasing in number and are more resistant to antifungal drugs [13]. *C. albicans* is the main agent accounting for 40-65% of the fungi isolated from *Candida* colonization cases. However, there is an increase in the incidence of other species, and the urinary tract now is more frequently colonized by non-*albicans* species notably *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. High incidence of candidiasis caused by non-*albicans* species were previously reported [14]. In this study, 30% of patients had colonization. But 20% of diabetic patients had infection which could be due to their increased tissue glucose level, facilitating the growth of *Candida*. However, infection rate was reported to be 14.3% by Seifi *et al.* [15].

The antifungal susceptibility patterns of *Candida* isolates showed more than 50% resistance against polyenes antifungals used (81.7% and 53.3% for AMB and NYS respectively). The highest resistance of AMB was observed against *C. krusei, C. glabrata* and *C. parapsilosis* with respective percentages of 100%, 92.9% and 75%. Our result is consistent with those obtained by Ruan *et al.* [16] regarding the susceptibility of *Candida* isolates to Amphotericin B. It is one of the most toxic antimicrobial agents in clinical use, although still qualifies as a standard treatment. Resistance has also been reported in non-*albicans Candida* species, for example, species *C. lusitaniae* and *C. guilliermondii* [17]. A total of 48.3% and 45% resistance were observed against ketoconazole and fluconazole.

The susceptibility of *Candida* species to frequently used antifungal drugs presents various levels. It has been reported that non-*albicans* species, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* have shown higher resistance rates against fluconazole than *C. albicans* [18]. As it could be expected, in our study, none of the *Candida* species identified was sensitive to terbinafine while 63.3% exhibited intermediate susceptibility. Terbinafine is a synthetic antifungal drug with fungicidal activity against dermatophytes, moulds and fungistatic activity against

Candida species [19]. Unlike results obtained by Salehei *et al.* [20] who obtained good results for terbinafine against *Candida* spp. isolates, several authors have reported that this drug does not present significant in vitro activity against *Candida* yeasts [21].

CONCLUSION

The prevalence of Candiduria in the population studied was 33.3% therefore the presence of candiduria in diabetic patients should not be neglected. Although *C. albicans* was the organism most involved, other *Candida* species were also isolated as clinically important opportunistic pathogens. This result reinforces the emerging importance of non-*albicans Candida* species in the epidemiology of candiduria. All the *Candida* species identified presented high level of resistance towards polyenes antifungals nystatin and amphothericin B. Therefore, the species identification of *Candida* isolates along with their antifungal susceptibility pattern can help the clinicians in better treating the patients with candiduria.

Table 4: Susceptibility patterns of *Candida* isolates to ketoconazole, fluconazole, nystatin, amphotericin B and terbinafine.

Antifungals		***Ca*** (n=19)	***Ck*** (n=14)	***Cg*** (n=12)	***Cp*** (n=10)	***Ct*** (n=3)	***Cd*** (n=2)	**Total**
	S	2 (10.5%)	8 (57.1%)	7 (58.3%)	6 (60)	2 (66.7%)	1 (50%)	26 (43.3%)
Ketoconazole	I	1 (5.3%)	0 (0.0%)	2 (16.7%)	2 (20)	0 (0%)	0 (0%)	5 (8.3%)
	R	16 (84.2%)	6 (42.9%)	3 (25.0%)	2 (20)	1 (33.3%)	1 (50%)	29 (48.3%)
	S	6 (31.6%)	9 (64.3%)	4 (33.3%)	4 (40)	0 (0%)	1 (50%)	24 (40.0%)
Fuconazole	I	2 (10.5%)	1 (7.1%)	3 (25.0%)	1 (10)	2 (67%)	0 (0%)	9 (15.0%)
	R	11 (57.9%)	4 (28.6%)	5 (41.7%)	5 (50)	1 (33%)	1 (50%)	27 (45.0%)
	S	4 (21.1%)	3 (21.4%)	3 (25.0%)	3 (30)	0 (0%)	0 (0%)	13 (21.7%)
Nystatin	I	2 (10.5%)	6 (42.9%)	4 (33.3%)	2 (20)	0 (0%)	1 (50%)	15(25.0%)
	R	13 (68.4%)	5(35.7%)	5 (41.6%)	5 (50)	3 (100%)	1 (50%)	32 (53.3%)
	S	2 (10.5%)	1 (7.1%)	0 (0.0%)	0 (0)	0 (0%)	0 (0%)	3 (5.0%)
Amphotericin B	I	5 (26.3%)	0 (0.0%)	3(25%)	0 (0)	0 (0%)	0 (0%)	8 (13.3%)
	R	12 (63.2%)	13 (92.9%)	9 (75%)	10 (100)	3 (100%)	2 (100%)	49 (81.7%)
	S	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0)	0 (0%)	0 (0%)	0 (0.0%)
Terbinafine	I	13 (68.4%)	11 (78.6%)	8 (66.7%)	5 (50)	0 (0%)	1 (50%)	38 (63.3%)
	R	6 (31.6%)	3 (21.4%)	4 (33.3%)	5 (50)	3 (100%)	1 (50%)	22 (36.6%)

Ca: Candida albicans, *Ck: Candida krusei, Cg: Candida glabrata, Cp: Candida parapsilosis, Cd: Candida dubliniensis,* S: susceptible, I: intermediate, R: resistant

Acknowledgements

JP Dzoyem is thankful to "The World Academy of Sciences (TWAS) for the advancement of science in developing countries" and to Committee on Scientific and Technological Cooperation (COMSTECH) for the (COMSTECH)-TWAS Joint Research Grants Programme awarded to provide equipment used for this work.

Author contributions

ISY and JPD conceived and designed the experiments; NDA and NC performed the experiments; ISY, CN and RTT analyzed the data; ISY and NDA drafted the manuscript and JPD finalized the paper.

Conflicts of interest

The authors declare no conflict of interest.

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