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Speciation and Anti-fungal Susceptibility Profile of *Candida* species isolated from Urine of Hospitalised Patients in Murtala Muhammad Specialist Hospital, Kano, Nigeria

Bashir, A. U. [1], Mohammed, Y.[1], Haruna, M. M.[2], Sharif, A. A.[1], Getso, M. I.[1], Abbas, M. A.[1], Yusuf, M. A.[1], Kabuga, A. I.[1], Daneji, I. M.[1], Aliyu, M.[1], Takalmawa, H. U.[1] and Adamu, A. Y.[1*]

[1] Department of Medical Microbiology and Parasitology,
Faculty of Clinical Sciences,
Bayero University Kano.

[2] Department of Community Medicine,
Faculty of Clinical Sciences,
Bayero University Kano.

Email: almukya@gmail.com

Abstract

Candiduria is a very common occurrence in patients with bladder colonization and in patients with upper urinary tract infections caused by retrograde transmission from the bladder or haematogenous spread from a distant source. The majority of people who have candiduria have no symptoms hence the rate of complications is unknown, although it appears to be minimal. Candidemia rarely develops from asymptomatic candiduria unless there is an obstruction or the urinary tract is instrumented. Unfortunately, there are no reliable diagnostic tests that can tell the difference between infection and colonization. This study aimed to determine the species distributions and antifungal susceptibility profile of Candida species among hospitalised patients with urinary tract infection in Murtala Muhammed Specialist Hospital, Kano. A systematic random sampling was used in this cross-sectional study to collect 150 urine samples. The urine samples were cultured on Sabouraud dextrose agar and Blood agar, isolates were identified using morphological appearance, germ tube test and biochemical reactions using API 20CAUX. Antifungal susceptibility testing of the isolates against amphotericin B, fluconazole and itraconazole was done using Agar Disc diffusion method. The prevalence of Candida species was 8.0%; Candida albicans (4/12), was the predominant specie isolated followed by C. famata (3/12), C. tropicalis (2/12), C glabrata (2/12), and C. parapsilosis (1/12). All the Candida isolates were susceptible to amphotericin B. Candida famata and C. parapsilosis were susceptible to fluconazole while half of C. glabrata and C. tropicalis isolates were susceptible to Itraconazole. The existence of intrinsic factors that lead to secondary resistance to antifungal agents needs to be hampered as this can lead to clinical resistance. Proper identification of fungal species and antifungal susceptibilities should be conducted for accurate management of inpatients.

Keywords: Antifungal, Candida, Candiduria, Resistance, Susceptible

Introduction

Candiduria occurs particularly in hospitalised patients, especially those in intensive care units, who often have multiple predisposing factors such as diabetes, indwelling urinary catheters, antimicrobial exposure, cancer, long hospitalization, gender and age (Kauffman *et al*., 2011). Many virulence factors aid in the transition of *Candida* species from commensals to

*Author for Correspondence

pathogens, including enhanced adhesion to host tissue and medical devices, biofilm formation and production of extracellular hydrolytic enzymes (Sardi *et al.,* 2013).
According to several studies, *Candida* species caused at least 10–15% of hospital-acquired Urinary Tract Infections (UTIs) and had increased over times (Kauffman *et al.,* 2000; Olusegun-Joseph & Killaney, 2016). Improperly diagnosed and treated candiduria due to *C. albicans* and non-*albicans Candida* species, can lead to morbidity and mortality (Deorukhkar & Saini, 2014). Many studies have documented various *Candida* species to be responsible for candiduria, Passos *et al.* (2005) reported *Candida albicans* as the predominant specie isolated from urine of patients in their study. They also found other species to include *C. glabrata*, *C. kefyr, C. parapsilosis, C. famata, C. guilliermondii, C. krusei* and *C. tropicalis*. Sumana *et al.* (2017) also isolated *Candida* species from urine samples, where *C. tropicalis* and *C. albicans* were the predominant followed by other non *albicans* including *C. glabrata, C. parapsilosis, C. famata. C. spherica, C. guilliermondii, C. krusei* and *C. haemulonii*. In a study conducted in Keffi, Nassarawa State, Nigeria at a tertiary institution, 56 *Candida* species were isolated from 144 urine samples examined. Most of the *Candida* were isolated from female students (46/56) while the remaining (10/56) were from the males. *Candida tropicalis* 24 (30%) was the predominant isolate among the female students, while *C. utilis,* 5 (7.8%) and *C. albicans* 5 (7.8%) were isolated among the male students (Tsaku *et al*., 2019).

Antifungal susceptibility testing studies revealed that most *Candida* species are susceptible to antifungal agents. Baran *et al*. (2000) tested the susceptibility patterns of 80 urine isolates of *Candida* species to fluconazole, amphotericin B and voriconazole and reported that all the isolates were sensitive to amphotericin B and voriconazole. However, *C. albicans* were the only isolates resistant to fluconazole with MICs ≥64µg/ml. In another study, majority of the *Candida* strains had low minimum inhibitory concentration (MIC) values to both fluconazole and amphotericin B. However, *Candida albicans* were susceptible to flucytosine and itraconazole, though resistance was reported to voriconazole and fluconazole as 5.45% and 3.63% respectively in *Candida albicans* (Tulumoglu *et al.* 2009). Sumana *et al.* (2017) tested all their isolates for antifungal susceptibility which revealed that 4% of *C. tropicalis, C. albicans, C. parapsilosis* and 2% of *C. glabrata* and *C. krusei* showed resistance to fluconazole. About 92% of the isolates were susceptible to voriconazole. Four percent of *C. parapsilosis* and 2% of *C. albicans, C. glabrata, C. tropicalis* and *C. haemulonii* were resistant to amphotericin B while ketoconazole resistance was found in 2% of *C. albicans* and *C. haemulonii*. Subira *et al.* (2018) observed 96.9% susceptibilities for micafungin and caspofungin in a study conducted in Kenya. Flucytosine was effective against all *Candida* isolates, while voriconazole and fluconazole were effective against 93.94% and 81.82% of the isolates.

In northern part of Nigeria, information on candiduria in hospitalised patients is plausible. This study aimed to determined species distribution and antifungal susceptibility profile of *Candida* species among hospitalised patients with urinary tract infections in Murtala Muhammad Specialist Hospital Kano.

Materials and Methods

Study Area, Design, Sample size and Ethical approval

The study was carried out at Murtala Muhammad Specialist Hospital (MMSH), Kano which was commissioned in 1926. Kano state is located in North-Western Nigeria with coordinates 11º 30 N 8º 30 E. It has a total area of 20,131km² (7,777sqm) and population of 11,058,300 (NPC, 2006). The study was a cross-sectional study where 150 hospitalised patients suspected of urinary tract infections were recruited. The sample size was estimated based on a previous

prevalence by Nwabuisi & Onile (2001) and either mid-stream or catherterised urine samples were collected from the consenting patients. Ethical approval was obtained from the research and ethics committee of the Kano State Ministry of Health (Reference No. MOH/OFF/797/T.I/1991).

Sample Collection and Processing

The urine specimen comprising of either midstream or catheter collection was collected into a wide-mouthed, sterile leak-proof screw cap container. They were immediately transported to the laboratory without any delay for further analyses (Mackie & McCartney, 1996). The urine samples were centrifuged and a loopful of the urine sediment was streaked onto a small area of the Sabouraud Dextrose Agar (amended with chloramphenicol and gentamicin) and Blood Agar. The inoculating loop was then used to spread the inoculum from the pool. All inoculated SDA and Blood Agar plates were incubated at 37°C for 24-48hrs and then read, colonies that were cream-colored, pasty and smooth were gram stained (Badiee *et al*., 2010).

Identification of Yeast Isolates

Germ tube test was done by transferring a loopful of the colony of yeasts into human serum in a labelled test tube and emulsified. The mixture was incubated at 37° for about 3 hours. A drop of the suspension was placed on a microscope slide and was examined under a microscope for germ tubes on the yeasts using the X10 and X40 objective lenses. Isolates producing germ tubes were presumptively identified as *Candida albicans* or *Candida dubliniesis* (Mackie & McCartney, 1996; Chander, 2009). Both germ tube positive and negative isolates were subjected to biochemical tests using Analytical profile index Auxillary (API 20 CAUX). The test was conducted using Biomerieux (France-08159H) test kit according to manufacturer's instruction and the numerical profile was identified through the website; www.apiweb.com.

Antifungal susceptibility test

Disc diffusion method using locally prepared antifungal single disc was carried out on the yeast isolates (Rasco, 2011). The antifungal solutions and required concentrations were prepared at the Drug and Manufacturing Unit of Aminu Kano Teaching Hospital, using fluconazole capsule (Diflucan), itraconazole capsule (Itacare) and amphotericn B powder (Amfocare) obtained from reliable pharmaceutical stores. Twenty microliter of the preparation was used to impregnate the blank Oxoid antimicrobial sensitivity testing discs. A loopful of the 2.0 McFarland Standard suspension of the colonies was aseptically streaked on Mueller Hinton agar containing 2% glucose and 0.5 µg/ml methylene blue (CLSI, 2008; Rasco, 2011). A sterile forcep was used to evenly distribute the appropriate antifungal discs; fluconazole (25µg), itraconazole (10µg) and amphotericin B (10µg). The plates were incubated aerobically at 37°C for 48hours. After incubation, a meter rule was used on the underside of the plate to measure the diameter of each zone of inhibition in mm (Cheesbrough, 2010). The results were recorded and compared with the zone diameter interpretive standards of Rasco (2011).

Results and Discussion

A total of 150 urine samples were collected comprising of 116 mid stream and 34 catheterised from the inpatients. The patients were made up of 81 males and 69 females with age range between 12 – 80 years and mean age of 18.5± SD 6.55years as shown on Table 1 and Figure 1 respectively.

Candida spp. was isolated from 12 of the 150 samples investigated in this study, resulting in 8.0 % prevalence of *Candida* in the samples. The number of isolates from catheterised urine (7) was significantly higher than that of mid-stream urine samples (5). The *Candida* spp isolated from catheterised samples were *C. albicans* (3), *C. famata* (3) and *C. parapsilosis* (1) while those from the mid-stream urine samples were *C. albicans* (1), *C. glabrata* (2) and *C. tropicalis* (2). Our findings have revealed that *Candida* implicated as a significant contributor to systemic infections and a common causative agent of nosocomial infections in inpatients which concurs with Michael & Diekiema (2010).

A prevalence of 8.0% for *Candida* spp isolated in both urine samples in hospitalised patients is far lower than that obtained in a study conducted in Brazil by Passos *et al.* (2005) where a prevalence of 44% was recorded. They also reported that *Candida albicans* was the predominant yeasts species isolated from the urine samples. Other *Candida* species that were isolated in Passos *et al.* (2005) were *C. glabrata, C. kefyr, C. parapsilosis, C. famata, C. guilliermondii, C. krusei* and *C. tropicalis*. Similar studies also revealed *C. albicans* as the predominant species (Menza & Wanjiru 2013; Kali *et al.,* 2013).

Findings from this study revealed that most of the *Candida* isolates were susceptible to the three antifungal drugs (Amphotericin B, Fluconazole, and Itraconazole). *Candida albicans* isolates were fully susceptible to amphotericin B, followed by *C. glabrata* and *C. parapsilosis* while *C. famata* and *C. tropicalis* showed *intermediate* susceptibilities among the isolates. In the case of fluconazole, all isolates of *C. glabrata* and *C. parapsilosis* were found to be susceptible whilst *C. tropicalis* showed intermediate susceptibility. Three isolates [*C. albicans* (2) and *C. famata* (1)] were found to be resistant to fluconazole. Half of the isolates were resistant to itraconazole where all *C. parapsilosis* (1) and *C. albicans* (4) isolates showed resistance to the drug. However, *C. tropicalis* and *C. glabrata* showed complete and intermediate susceptibility respectively. Though, majority of the *Candida* isolates tested in this research are susceptible to the antifungal drugs investigated. However, there is a high tendency of increased antifungal resistance in life-threatening *Candida* infections especially if hospital acquired (Ingham *et al.,* 2012).

Similar findings were reported by Baran *et al*. (2000) where they evaluated the susceptibilities of 80 urine *Candida* isolates to fluconazole, amphotericin B and voriconazole in Michigan, United States and found out that all of the isolates were susceptible to amphotericin B and voriconazole.

Tulumoglu *et al.* (2009) reported the antifungal susceptibility of 48 *Candida* strains isolated from urine to flucytosine, amphotericin B, fluconazole, itraconazole, and voriconazole and reported that the resistance rate to voriconazole and fluconazole were 5.45% and 3.63% respectively in *Candida albicans*. They also reported that all *Candida albicans* were susceptible to flucytosine, itraconazole, and amphotericin B. Sumana *et al.* (2017) isolated 50 *Candida* spp from urine samples and reported that most of the *Candida* species were susceptible to fluconazole, voriconazole amphotericin B and ketoconazole. On the contrary, all of the isolates were susceptible to itraconazole. In Mombasa, Kenya a similar trend was recorded by Subira *et al.* (2018) where flucytosine was effective against all *Candida* isolates (100%), micafungin and caspofungin were effective against 96.9% of isolates, voriconazole was effective against 93.94% of isolates while fluconazole was effective against 81.82% of isolates.

Table 1: Demographic profile of the patients recruited for the study

Variables	Number	Percentage
Age		
10 – 19	15	10.0
20 – 29	26	17.3
30 – 39	31	20.7
40 – 49	20	13.3
50 – 59	19	12.7
60 – 69	12	8.0
70 – 79	17	11.3
>79	10	6.7
Total	**150**	**100**
Gender		
Male	81	54.0
Female	69	46.0
Total	**150**	**100**
Occupation		
Business	73	48.7
Artisan	5	3.3
Civil Servant	24	16.0
House Wife	38	25.3
Students	10	6.7
Total	**150**	**100**

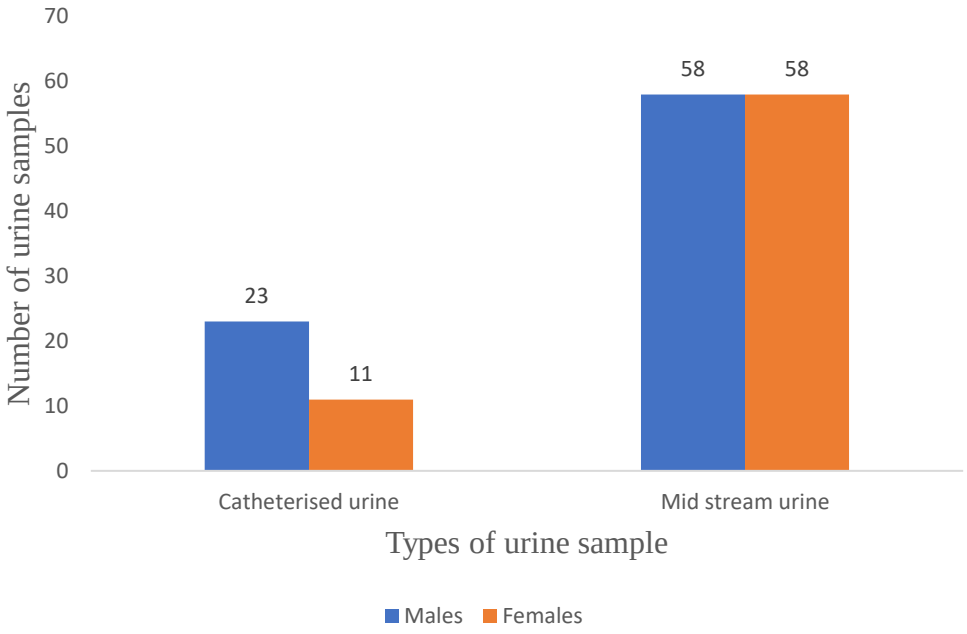

Figure 1: Distribution of types of urine samples collected from in-patients

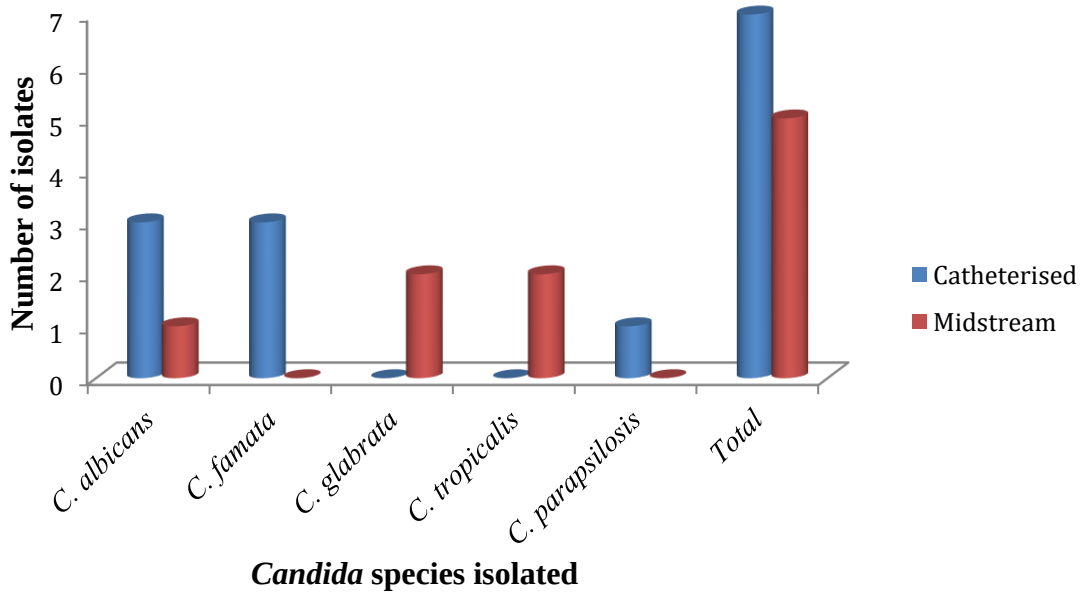

Figure 2: *Candida* species isolated from urine samples

Table 2: Antifungal susceptibility testing profiles for the *Candida* isolates

Candida spp	Amphotericin B			Fluconazole			Itraconazole		
	S	I	R	S	I	R	S	I	R
C. albicans	4	0	0	1	1	2	0	0	4
C. famata	1	2	0	2	0	1	0	2	1
C. glabrata	2	0	0	2	0	0	1	1	0
C. tropicalis	1	1	0	0	2	0	2	0	0
C. parapsilosis	1	0	0	1	0	0	0	0	1

Total	**9**	**3**	**0**	**6**	**3**	**3**	**3**	**3**	**6**

Key: S = Sensitive, I = Intermediate, R = Resistant

Conclusion

The prevalence of *Candida* species in urine samples collected from the patients at the Murtala Muhammad Specialist Hospital is 8.0%. The number of *Candida* spp isolates among catheterised urine samples was higher than that of midstream urine samples. *Candida albicans* and *C. famata* were the predominant *Candida* spp isolated from the specimens. All *Candida* spp isolated in this study are susceptible to amphotericin B. The existence of intrinsic factors that led to secondary resistance to antifungal agents needs to be hampered as this can lead to clinical resistance. Proper identification of fungal species and antifungal susceptibilities should be conducted for accurate management of inpatients.

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