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IN THIS ISSUE

- Candiduria
- Hypoglycaemia
- At-Risk Behaviour for Heart Diseases
- Healthcare-seeking Behaviours
- Post-Kidney Transplant Urine Bacteriology
- Osteonecrosis of Femoral Head
- Papanicolaou smear
- Mauriac syndrome
- Diabetic Ketoacidosis

ORIGINAL RESEARCH

Prevalence and Antifungal Susceptibility Pattern of *Candida species* Isolated from Patients with Urinary Tract Infections

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Abstract

Background: The presence of *Candida* species in the urine is termed candiduria, and it is a common form of urinary tract infection (UTI). Many other species of *Candida* organism apart from *Candida albicans* are known with increasing the occurrence of resistance to available antifungal agents.

Objectives: To determine the prevalence and sensitivity pattern of *Candida* isolates obtained from urine samples of diagnosed urinary tract infections.

Methods: Midstream urine of patients attending the Lagos University Teaching Hospital, Ikeja Lagos, were collected and inoculated on Sabouraud Dextrose Agar (SDA). Microbiological processing was done with Gram reaction, germ tube test, CHROME agar TM and sugar fermentation test using API 32C system. Antifungal susceptibility tests were done using the agar disc diffusion method.

Results: *Candida species* were obtained from 36 (12.9%) of 280 patients with UTI. *Candida albicans* (CA) had the highest frequency (12; 33.3%) compared to 24 (66.7%) for Non-albicans Candida (NAC). The speciation of *Candida* using Chrome Agar showed some misidentification from the API32C identification, but there was a significant correlation between API32C and Chrome Agar methods (r = 0. 9793). Half of the *C. albicans* species were sensitive to fluconazole while *C. hellenica* was only sensitive to Nystatin. The *C. parapsilosis* had the highest susceptibility pattern, with 86% and 71% for fluconazole and ketoconazole, respectively. Generally, ketoconazole had the highest effectiveness on *Candida* species.

Conclusion: This study demonstrated the role of *Candida* species in UTIs and their high susceptibility to ketoconazole.

Key words: Candida albicans, Candiduria, Non-albicans Candida, Urinary Tract Infections.

Introduction

Candiduria is known as the most frequent nosocomial fungal infection worldwide. *Candida albicans* is the most common cause of nosocomial fungal urinary tract infections. [1]

Over one hundred and fifty million people worldwide experience an episode of candiduria yearly. [2] The presence of yeast or *Candida* species in the urine is termed candiduria. It can be detected by methods such as microscopy showing pseudohyphae and budding of yeast cells and cultural and

genetic techniques. The risk factors include urinary tract instrumentation, surgical procedures, antibiotic use, advanced age, female gender, intensive care unit (ICU) admission, immunosuppressive therapy, and prolonged hospitalisation. [3]

The leading three species of *Candida* include *C. albicans, C. glabrata* and *C. tropicalis,* respectively. Genitourinary tract infections consist of different infections, including asymptomatic and symptomatic candiduria, cystitis, pyelonephritis, candida balanitis and vulvovaginal candidiasis. [1] The aetiology of candiduria changes with greater than 50% of urinary isolates caused by the Non-*albicans Candida* (NAC), and candiduria can be a marker for a serious underlying illness. [4]

However, rapid change in the role of *Candida* species in the causation of urinary tract infections in some countries has been noted. In Ghana, *C. glabrata* is dominant among the isolated *Candida* species relative to C. *albicans* [46 (27.7%) against 59 (35.5%)]. [5] In Nasarawa State, Nigeria, *C. tropicalis* was the most frequently isolated agent of candiduria, followed by *C. albicans* and *C. utilis,* respectively. [6]

Antifungal agents play a significant role in the treatment f of UTI. An increasing number of antifungal agents can be used to treat mycotic infections. [2, 7] The treatment of fungal diseases is compromised by the emergence of antifungal drug resistance, which was hitherto considered a rare occurrence. Currently, antifungal drug resistance is now on the rise. The evolution of multidrug-resistant organisms refractory to several different classes of antifungal agents is most concerning, especially among common *Candida* species. The resistance mechanisms include inherently reduced susceptibility and those acquiring resistance during therapy. [8]

The classes of the currently available drugs belong to the groups of polyenes, azoles and echinocandins. Polyenes and azoles act on the cell membrane and echinocandins disrupt the fungal cell wall. Other classes are ally amines and antimetabolites. [9] Recently, an increase in the number of *Candida* species resistant to antifungal drugs has been recognised worldwide; *C. tropicalis* and *C. pararapsilosis* are both generally susceptible to azoles, whereas *C. glabrata* and *C. krusei* are intrinsically more resistant to antifungal agents, particularly to Fluconazole. [8] Therefore, this study was conducted to determine the prevalence, and antifungal sensitivity pattern of *Candida* isolates obtained from urine samples of diagnosed urinary tract infections.

Methods

The study adopted a cross-sectional design, and the study involved consenting patients aged more than 16 years with the provisional diagnosis of UTI from July to December 2014. The sample size was determined using Fisher's formula:

$$N = \frac{t^2 \times p(1-p)}{M^2}$$

Where:
N = required minimum sample size.
t = confidence level at 95% (standard value of 1.96).
p = estimated prevalence of candiduria in patients with UTI in the project area.
M = margin of error at 5% (standard value of 0.05).

For this study:
$N = (1.96)^2 \times 0.2 \times (1 – 0.2)/ (0.05)^2$
N = 245.86
An additional 10% of this was added to adjust for attrition. Therefore, the final minimum sample size was 280.

Ethical approval for the study was obtained from the Lagos State University Teaching Hospital Health Research Ethical Committee, HREC, with reference number

LREC/10/06/303. Informed consent was also obtained from all the patients.

Mid-stream urine samples were collected from the 280 adult patients with the provisional diagnosis of UTI (symptomatic) attending Outpatient clinics at the Lagos State University Teaching Hospital, Ikeja. Excluded from the study were patients aged less than 16 years and those with no features of UTI. The urine samples were examined macroscopically for colour and turbidity, centrifugation was done at 1500rpm for five minutes, and wet preparation of the sediment was examined microscopically under X4 and X10 lenses. The urine samples were Gram-stained and cultured on Sabouraud Dextrose Agar (SDA), incubated at 37ºC for 24-48 hours. Colonies were read based on their colonial appearances, large size, shape, colour, elevation and consistency. The isolates were identified using Gram reaction, Germ tube test, and Chrome agar, while *Candida* biochemical identification was carried out using the API32C system. The Germ tube test was done by dispensing 0.5mls of human serum in Khan tubes and was incubated with five colonies of *Candida* isolates for three hours. One drop from the suspension was placed on a grease-free glass slide, and a drop of lactophenol cotton blue was added and examined for the formation of a germ tube. Sugar fermentation tests were also performed using the API 32C system. Purified isolate suspension was made to match turbidity equivalent to 2 McFarland standard, and 250μl was transferred into an ampoule of the API C medium. A volume of 135μl of the yeast basal medium suspension was pipetted into each freeze-dried sugar wells and incubated at 30ºC for 24 hours. Visual turbidity indicated growth, while lack of turbidity indicated no growth. The result was correlated with the API 32C identification software. The isolates were aseptically inoculated on Chrome agar and were incubated at 37ºC for 48 hours. According to the manufacturer's instructions, the colonies were then identified based on colours.

Antifungal susceptibility tests were done using the agar disc diffusion method. The inoculum suspension was prepared and matched with 0.5 McFarland standard. The standardised suspension was flooded on the surface of the plate, the excess was drained, and antibiotic discs were placed and incubated at 37ºC for 24-48 hours. Antifungal discs used included Itraconazole (8μg), Ketoconazole (10μg), Fluconazole (25μg), Terbinafine (30μg), Griseofulvin (25μg) and Nystatin (100μg). The zones of inhibition were measured and interpreted using the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. [10] The Control strain used was *Candida albicans* ATCC 10231.

The data were analysed using the Statistical Package for Social Sciences (SPSS) Windows version 21.0. The socio-demographic characteristics of the participants were presented using frequency tables. Continuous variables were summarised using descriptive statistics such as the mean and standard deviation at 95% Confidence Interval (CI). The level of statistical significance was set at $p < 0.05$.

Results

Out of 280 urine samples, there were 121 (43.2%) males and 159 (56.8%) females (Table I). A total of 36 species of *Candida* were isolated, representing 12.9% of all the patients with UTI. These 36 isolates were obtained from the urine samples of 10 (27.8%) males and 26 (72.2%) females. Most of the isolates were obtained from people in the age group of 31-40 years (8; 22.22%), while the lowest yield was obtained at age >80 years (1; 2.78) (Table II).

Table I: Age and sex distribution of patients with Urinary Tract Infections

Age Group (Years)	Sex		Total
	Male	**Female**	
16-20	14 (44.0)	14 (56.0)	28 (8.9)
21-30	23 (46.0)	27 (54.0)	50 (17.9)
31-40	19 (29.7)	45 (70.3)	64 (22.9)
41-50	11 (30.6)	25 (69.4)	36 (12.9)
51-60	14 (38.9)	22 (61.1)	36 (12.9)
61-70	26 (66.7)	13 (33.3)	39 (13.9)
71-80	10 (45.5)	12 (54.5)	22 (7.8)
>80	7 (87.5)	1 (12.5)	8 (2.7)
Total	**121 (43.2)**	**159 (56.8)**	**280 (100.0)**

$X^2 = 23.145$; p = 0.001

Table II: Age and sex distribution of patients with Candiduria

Age Group (Years)	Sex		Total
	Male	**Female**	
16-20	1 (33.3)	2 (66.7)	3 (8.3)
21-30	1 (20.0)	5 (80.0)	6 (13.9)
31-40	3 (37.5)	5 (62.5)	8 (22.2)
41-50	2 (33.3)	4 (66.7)	6 (16.7)
51-60	1 (20.0)	4 (80.0)	5 (13.9)
61-70	1 (20.0)	4 (80.0)	5 (13.9)
71-80	1 (33.3)	2 (66.7)	3 (8.3)
>80	0 (0.0)	1 (100.0)	1 (2.8)
Total	**10 (27.8)**	**26 (72.2)**	**36 (100.0)**

Candida albicans (CA) had the highest prevalence (33.3%). Non-albicans Candida (NAC) included C. *tropicalis* (25.0%), C. *parapsilosis* (19.4%), C. *grabrata* (5.6%), *C. hellenica* (2.8%) *and S. cerevisiae* (2.8%) as shown in Table III. The speciation of *Candida species* using Chrome Agar showed some misidentification from AP I32C results in 6 (16.7%) cases. NACs (*C. tropicalis, C. parapsilosis, C. krusei, C. kefyr and C. glabrata*) were misidentified. All CA were identified correctly (Table IV). Figure 1 shows the correlation between API 32C and Chrome Agar methods of speciation, and there was a significant correlation between API 32C and Chrome Agar methods (r = 0.9793).
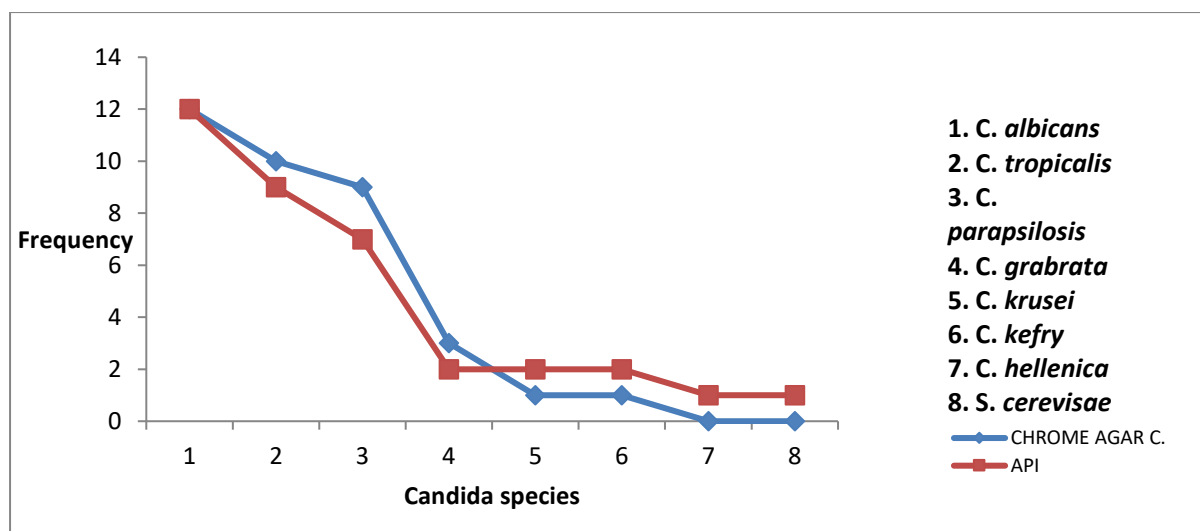
Table V shows that all the *Candida* species isolated were resistant to griseofulvin; only 50% of *C. albicans* were sensitive to fluconazole, and *C. hellenica* was only sensitive to Nystatin. *Candida glabrata* had the highest susceptibility pattern to itraconazole and ketoconazole with 50% and 100% susceptibility rates, respectively. *C. parapsilosis* had the highest susceptibility rates (86% and 71%) to fluconazole and ketoconazole, respectively. *C. albicans* had 50% susceptibility to fluconazole but 25% susceptibility to itraconazole. Ketoconazole had the highest effectiveness on *Candida* species with activities against six *Candida* species.

Table III: Distribution of *Candida* species isolated from patients with urinary tract infections.

Candida species	Frequency	Percentage
C. albicans	12	33.3
C. tropical	9	25.0
C. parapsilosis	7	19.4
C. grabrata	2	5.6
C. crusei	2	5.6
C. kefry	2	5.6
C. hellenica	1	2.8
Saccharomyces cerevisiae	1	2.8
Total	**36**	**100**

Table IV: Identification of *Candida* species using the Chrome Agar and API 32C methods

Candida species	Chrome Agar	
	Candida	API 32C
C. albicans	12	12
C. tropical	10	9
C. parapsilosis	9	7
C. grabrata	3	2
C. crusei	1	2
C. kefry	1	2
C. hellenica	0	1
Saccharomyces cerevisiae	0	1
Total	**36**	**36**

1. C. *albicans*
2. C. *tropicalis*
3. C. *parapsilosis*
4. C. *grabrata*
5. C. *krusei*
6. C. *kefry*
7. C. *hellenica*
8. S. *cerevisae*

CHROME AGAR C.
API

r = 0.9793.

Figure 1: Correlation between API 32C and Chrome Agar methods

Discussion

Urinary tract infection is one of the microbial infections affecting all age groups worldwide, and candiduria is a common fungal form of UTI. In the present study, most of the patients presenting with clinical features of UTI were females. This finding agrees with the previous work of Josiah *et al.* in Calabar, Nigeria, who reported a higher incidence of UTI among

females than males. [11] The sex-related predominance could be due to the shorter length of the female urethra and its closeness to the anus.

The present study showed candiduria in 12.9% of patients with UTI in agreement with the report of Gharaghani *et al.* in 2018, which showed that candiduria in 10-15% of UTI cases. [12] The prevalence of candiduria was higher among females compared with males. This pattern is similar to the finding of Yismaw *et al.*, that significant candiduria was strongly associated with the female sex. [13] The sex distribution of the 36 *Candida species* isolated in the present study showed female preponderance similar to the findings in a survey carried out in Keffi, northern Nigeria where the prevalence among female students was four times higher than among the males. [6]

C. albicans recorded the highest incidence rate of 33.3% among the species of *Candida*

isolated. This rate is relatively lower compared with the report of Akortha *et al.*, which recorded a prevalence of 63.9% for C. *albicans* in Benin City. [14] The result of the present study is in contrast with the findings of Okungbowa *et al.*, who reported C. *glabrata* as the most common *Candida* species among symptomatic individuals in Nigerian cities. [15] Also, in this study, 67.7% of the cases were non-albicans *Candida* species, different from the reports from Hollandia *et al.*, with an overall prevalence of non-albicans *Candida* of 21% and of which 89% were C. *glabrata*. [16] These variations in the report may be attributed to the method used in the identification and differences in population types. [17] However, the findings in the present study are similar to a Turkish with prevalence rates of 44%, 18% and 20% for *C. albicans*, C. *glabrata* and C. *tropicalis*. [18] Furthermore, a study in Iran reported a prevalence of 77.4% for *Candida albicans*. [19]

Table V: Susceptibility pattern of Candida species to selected antifungal agents

Antifungal Agent		C. albicans	C. glabrata	C. parapsilosis	C. krusei	C. kefry	C. hellenica	C. tropicalis	S. cereviasea	C. albicans ATCC 1021
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Itraconazole	S	3 (25.0)	1 (50.0)	2 (28.6)	0 (0.0)	1 (50.0)	0 (0.0)	2 (22.2)	1 (100.0)	0 (0.0)
	I	6 (50.0)	1 (50.0)	1 (14.3)	2 (100.0)	0 (0.0)	0 (0.0)	4 (44.5)	0 (0.0)	1 (100.0)
	R	3 (25.0)	0 (0.0)	4 (57.1)	0 (0.0)	1 (50.0)	1 (100.0)	3 (33.3)	0 (0.0)	0 (0.0)
Nystatin	S	2 (16.7)	0 (0.0)	3 (42.9)	1 (50.0)	0 (0.0)	1 (100.0)	1 (11.1)	0 (0.0)	0 (0.0)
	I	6 (50.0)	2 (100.0)	3 (42.9)	0 (0.0)	1 (50.0)	0 (0.0)	3 (33.3)	1 (100.0)	1 (100.0)
	R	4 (33.3)	0 (0.0)	1 (14.2)	1 (50.0)	1 (50.0)	0 (0.0)	5 (55.6)	0 (0.0)	0 (0.0)
Ketoconazole	S	7 (58.3)	2 (100.0)	5 (71.4)	1 (50.0)	0 (0.0)	0 (0.0)	5 (55.6)	1 (100.0)	1 (100.0)
	I	2 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	2 (22.2)	0 (0.0)	0 (0.0)
	R	3 (25.0)	0 (0.0)	2 (20.0)	1 (50.0)	1 (50.0)	1 (100.0)	2 (22.2)	0 (0.0)	0 (0.0)
Fluconazole	S	6 (50.0)	1 (50.0)	6 (85.7)	0 (0.0)	1 (50.0)	0 (0.0)	2 (22.2)	0 (0.0)	1 (100.0)
	I	3 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (55.6)	0 (0.0)	0 (0.0)
	R	3 (25.0)	1 (50.0)	1 (14.3)	1 (100.0)	1 (50.0)	1 (100.0)	2 (22.2)	1 (100.0)	0 (0.0)
Terbinafine	S	1 (8.3)	1 (50.0)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	1 (100)	0 (0.0)
	I	6 (50.0)	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	5 (41.7)	1 (50.0)	4 (57.1)	2 (100)	2 (100)	1 (100)	8 (88.9)	0 (0.0)	1 (100)
Griseofulvin	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	12 (100.0)	2 (100.0)	7 (100.0)	2 (100.0)	2 (100.0)	1 (100.0)	9 (100.0)	1 (100.0)	1 (100.0.0)

ATCC- American Type Controlled Culture, S- Sensitive, R-Resistant, I- Intermediate.

Several chromogenic substrates-containing media have been developed to facilitate the rapid identification of Candida species.

Chromogenic media are frequently used in the direct and indirect identification of yeasts because of the unique colour produced on

these media by *Candida* species. Chrom Agar *Candida* was employed to differentiate *Candida* species by colour and morphology. The API 32C method enabled the correct identification of isolates. The visual interpretation might sometimes be different and require expertise than in a routine clinical laboratory.

In this study, *Candida* species had the highest susceptibility rate to ketoconazole, effective against six *Candida* species. This is inconsistent with the report of Akortha *et al.*, which observed high susceptibility to Fluconazole among *Candida* species. [14] Nystatin, itraconazole and ketoconazole showed an excellent pattern of effectiveness against *Candida* species in agreement with the findings of Al-abeibe *et al.,* which reported that *Candida* species were susceptible to Nystatin, itraconazole and ketoconazole. [20]

All the *Candida* species isolated in the present study were resistant to griseofulvin. Half of *C. albicans* isolated were sensitive to fluconazole, in agreement with the finding of Ekpo *et al.*, in Cameroon [21] but *C. hellenica* was only sensitive to Nystatin. The *C. grabrata* had the highest sensitivity rate to intraconazole and ketoconazole. The *C. parapsilosis* had susceptibility rate of 86% and 71% to fluconazole and ketoconazole respectively. Overall, *Candida* species had the highest susceptibility rate to ketoconazole.

Conclusion

Candida albicans was a significant cause of candiduria in a population of adults with UTIs. The speciation of *Candida* using Chrome agar showed great benefit, but API 32C was better. *Candida* species showed various degrees of susceptibility patterns to different antifungal agents. Therefore, speciation and susceptibility testing for fungal isolates should be employed routinely in laboratories.

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