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Detection and Antimicrobial Susceptibility of *Candida* Species Isolated from the Urine of Patients in a Tertiary Health Facility, Southwest Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to identify and determine the antimicrobial susceptibility profiles of *Candida species* causing UTI among patients attending a tertiary Teaching Hospital, in southwest Nigeria.
Study Design: Comparative cross-sectional study.
Place and Duration of Study: Lagos State University Teaching Hospital, Ikeja, Nigeria between June 2017 and February, 2018.

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Methodology: A total of 250 participants whose provisional diagnosis was candiduria were recruited for this study. Urine samples were collected from consenting participants early in the morning into sterile wide mouth universal containers. These samples were cultured aerobically on Blood agar, Cystine-Lactose-Electrolyte Deficient (CLED) agar and Sabouraud dextrose agar (SAB) at 370C within 1 to 2 days. The isolates were profiled into species level using microscopic, biochemical test, chromogenic media (Chrom agar Candida) and Analytical Profile Index (API) 32C examination analysis.

Results: An overall rate for Candidiasis in this study was 12.8% (32/250). The rate was higher in female 17.5% compared to 6.5% in their male counterparts (p=0.014). Highest rate of infections peaked at 28.6% among age group 83-92 years and lowest (6.7%) in age group 23-32 years. A total of 11 (4.4%) urine culture from participants produced C. albicans pure fungi isolates. However, by gender, this was statistically significant (p=0.003). A total of 81 bacterial (32.4%) and fungal isolates 32 (12.8%) were isolated and profiled. Distribution of Candida species indicated highest incidence in age range 31-40 years, followed by 21-30years and age 61-70 years age brackets. The isolated species were Candida albicans 11(34.4%), *C. tropicalis* 8(25%), *C. parapsilopsis* 6(19%), *C. krusei* 2(6.3%) and *C. hellenica* 1(3.1%). Sixty to seventy percent of fungal isolates were susceptible to ketoconazole and fluconazole while the susceptibility pattern of Candida species to itraconazole, terbinafine and nystatin varied between moderately susceptible to resistance. All the Candida isolates were resistant to griseofulvin. However, C. albicans was found to be the major Candida species causing Candidal urinary tract infection. The only *C. hellenica* isolated was resistant to all the antifungal drugs were approved except nystatin.

Conclusion: High profile of Candida isolates and related UTI microbes were found in this study. There is need for advocacy for the use of API and Chrom agar Candida for routine diagnosis for Candida identification. Nevertheless, the Candida isolates were mostly susceptible to ketoconazole and Fluconazole but only the isolates were griseofulvin resistant.

Keywords: Candida; antimicrobial susceptibility; sabouraud dextrose agar; antifungal disc; Nigeria.

1. INTRODUCTION

"The of candida species identification in the urine of patients is indicative of colonization with ultimate urinary tract infection (UTI) referred to as candiduria" [1]. "However, the causative agent of this clinical condition varies according to the geographic region, study period, and type of healthcare facility. *Candida albicans* is the most prevalent candiduria agent; however, *Candida* non-*albicans* (CNA) species have been reported worldwide which calls for serious health concerns" [2, 3]. "Candida species in measurable quantities in the urine (Candiduria) are found in less than 1% of clean voided specimens in healthy persons, but account for 5% of all urine cultures results in the general hospital setting and 10% of urine isolate in tertiary care facilities. Majority of Candida species have the capacity to cause UTI, while in major centers globally non *Candida albicans* species are prevalent" [4]. "Candida species are unusual cause of UTI in healthy individual but common in the hospital setting or among patients with predisposing diseases and structural abnormality of the kidney and duct structure" [5]. "In severe systemic episodes of *Candida* infections, management of clinical conditions and enhanced patient survival depend on rapid interventions. Thus, correct identification of the pathogen and administration of specific antifungal therapies are crucial for patient recovery" [6,7,8].

"The isolation of *Candida* spp. from urine cultures may indicate colonization or urinary tract infection (candiduria), but it may also be a sign of severe systemic candidiasis or candidemia" [6,9]. "*Candida albicans* is the commonest fungus of medical importance. It can be found everywhere in our environment and could be passed directly form one person to another" [3,4]. "*C. albicans* is a commensal and constituent of the normal gut flora comprising microorganisms that live in the human mouth and gastrointestine tract. It is found in 80% people without harm, while candidiasis is a consequence of its multiplication" [1,10]. "Candida growth is under control as a result of the presence of other bacteria in our body. However, if the bacteria balance is compromised symptoms will arise. Candida normally causes infection in warm and moist areas" [11]. "Candidiasis can stem from overuse of antibiotics. When antibiotics are prescribed to eradicate injurious bacteria, a lot of friendly flora, for example, acidophilous and bifidous organisms in both intestines are destroyed"

[12,13].*Candida* spp. can reach the urinary tract via the ascending route, from the urethra to the bladder, or by hematogenous spread, as *Candida* spp. is filtered by the kidneys and excreted in the urine.

"The *C. albicans* as an organism frequently colonizes the oropharynx, colon and vagina of healthy humans and can enter the urinary tract by ascending from the perineum (retrograde infection)or by hematogenously seeding the kidney and " spilling over" into the urine (antegrade infection)" [12,14]. "These organism species poorly attach to the mucosa of the bladder through the obstruction of urinary tract, bacteriuria concomitant or pronounced invasion while immunosuppression of the wall of the bladder, ureter and/or kidney can ultimately take place" [15]. "Most patients with candiduria are asymptomatic" [6]. Yeast can be detected in urine that is contaminated during collection, in patients who have upper urinary tract infection that developed either from retrograde spread from the bladder or spread from a distant source.

"Candiduria occurs much less commonly in adult patients in critical care facilities than in infants where candiduria represent colonization and antifungal therapy is not required" [16]. "Some small observational studies have found that premature infants who have candiduria, or yeast in the urinary tract, are more likely to have a widespread infection with a high risk of death or impairment of brain development in children who survive. Nevertheless, healthcare managers don't ascertain candiduria as a result of sample contamination bias results" [15,16]. "Contamination can usually be differentiated from urinary tract colonization or UTI by obtaining and culturing new urine samples to see if yeast persists. In older women, to eliminate contamination by perineal flora, it is necessary to obtain the second urine specimen by sterile bladder cauterization. If the second specimen yields no yeast on culture, it can be assumed that contamination by perineal flora was the cause of candiduria, and no further diagnostic studies are needed" [12,16].

"Most UTIs due to Candida or episodes of candiduria occur in hospitalized patients with indwelling bladder catheter" [17]. "It is common in intensive care units (ICUs) and may represent the most frequent UTIs encountered in adult surgical ICU" [18]. "In United States, the percentage of nosocomial UTIs due to candida species increased from 22% for the period 1986-1989 to almost 40% for the period 1992-1997" [19]. "Yeast related UTI are rare in healthy newborns. Candiduria is also reported to complicate urological surgery following the placement of prosthetic devices for major congenital urological malformations" [16]. "Consequently, candiduria which signifies the presence candida species in urine and data of its global preponderance are available" [20]. "However, investigating both non-*Candida albicans* species and *Candida albicans* indicates that the former is more frequent in urinary tract with more than 50% of urinary isolates of Candida belonging to non albicans species while others have identified *Candida glabrata* as the dominant species". [21] *"C. glabrata* apparently adapts well to selected urine properties such as substrate availability, osmolarity and pH" [22]. "For many years *C. albicans* was the most prevalent species isolated from the urinary tract. Higher death rate of victims of candiduria is on the rise as against those who do not have candida in their urine" [23,24].

2. METHODOLOGY

2.1 Study Area

Lagos State University Teaching Hospital, Ikeja, is located in the northern part of metropolitan Lagos and draws its patients from all over the state (Lagos State Website) is owned by the state government as a tertiary health facility for the state University. Ikeja is an outer-ring suburb of the city of Lagos and the capital of Lagos State. It lies 20 km north of Lagos Island. It is situated at 6.59° North latitude, 3.34° East longitude. Lagos state is located on the south-western part of Nigeria, it lies approximately on longitude 3^0 23'45" east and between latitude 6^0 27'11"N. The population of Lagos is about 20 million with an area of 356,861 hectares with a growth rate of 3.2%. Lagos was the former capital of Nigeria and is the most economically viable city in Nigeria in which all tribes from all the country concentrate in it as a business hub having this massive population of 20million inhabitants. It has as much as 37 local governments distributed across land and water.

2.2 Participants

All participants who willingly indicated their interest after being suspected of UTI and sent to LASUTH Medical Microbiology Laboratory for microscopic analysis, sensitivity and culture were

recruited into the study. Consenting patients (participants presenting with provisional diagnosis of urinary tract infection (symptomatic and asymptomatic), clinical history of being immunocompromised and from the age of 13 years and above were considered. However, patients with no case of UTI and of age 12 years and below were not recruited and excluded from the study.

2.3 Sample Collection

Consenting patients were informed to carefully void early morning (midstream) urine into sterile transparent universal containers using aseptic technique and brought for submission to the laboratory. Each sample was properly identified with the name of each participant, date and time of collection and kept in a refrigerator or in an ice pack box before culturing. The hospital form for patient data collection contains all relevant information required for this study investigation.

2.4 Laboratory Analysis

Urine samples were cultured aerobically on Blood agar, Cystine-Lactose-Electrolyte Deficient (CLED) agar and Sabouraud dextrose agar (SAB) at 37^0C within 1 to 2 days. The isolates were profiled into species level using microscopic, biochemical test, chromogenic media (Chrom agar Candida) and Analytical Profile Index (API) 32C examination analysis.

2.4.1 Culture media

Sabouraud dextrose agar (pH 5.6) containing corn meal extract was prepared to give a final pH (at 25°C) 6.0 which will enhance growth and preserve the chlamydiospores according to the manufacturer's instructions. Bacteria stool culture media (Selenite F, MacConkey, DCA, SS agar) was prepared according to manufacturer's instruction and used to detect or exclude bacterial isolates. As for inoculation, with the use of sterile wire loop and streaking method to obtain discreet colonies on culture plates, all stool samples including control were inoculated into two different sterile freshly prepared Sabouraud dextrose agar plates containing corn meal extracts and antibiotics (chloramphenicol, streptomycin, gentamycin at a concentration of 100mg/liter of medium respectively) because of the bacteria load in stool specimens and also cultured on the different routine bacteria stool culture media mentioned above to identify and then exclude gastroenteritis of bacteria origin. All inoculated media including the Selenite F broth was incubated at 35°C – 37°C respectively. One of the Sabouraud agar plate were incubated at room temperature (18°C – 25°C).

2.4.2 Plate reading

Presumptive fungi colonies were read based on their colonial appearance: size, shape, elevation, consistency (mucoid, dry or semi-mucoid), colour or pigmentation, and distinctive yeast (bread) smell. The number of colonies on plates showing pure fungal growth was counted. Those with counts ranging from ≤103, 104 and ≥105 cfu/g after 24 - 72hrs incubation were regarded as significant. Plates that showed no growth after 7days incubation on the Sabouraud dextrose corn meal extract agar plates were disregarded and discarded appropriately. Bacterial isolates from the routine bacteriology culture plates were identified and their pathogenicity determined appropriately.

2.4.3 Identification test methods

These are test procedures that were used for the identification and characterization of the various fungal species isolated. The tests include Gram's reaction. Smears of isolates were made on grease free slides, stained by Gram's Method and examined under the x100 objective of the microscope. Yeast cells stained purple blue indicating that they were Gram positive. Gram negative organisms stain red. As for Germ tube test, suspension of pasty colonies of white or cream color on fungal culture plates were made in khan tubes containing 0.5 ml of human serum, incubated for 2 to 3hours at 37°C and examined microscopically (a drop of lacto phenol cotton blue was added to the drop of the suspension on the slide to stains the yeast cells) for the detection of yeast cells with sprouting /tubelike outgrowth from the cells (germ tubes). *C. albicans* is germ tube positive.

2.4.4 Preliminary speciation of candida using special selective fungal agar media CHROMagarTM

Suspected isolates were subcultured on BBL™ CHROMagarTM. The inoculated plates were incubated aerobically at 37°C for 24 hours. After incubation, the colours of the isolates were noted and identified on the colour chat.

2.4.5 Sugar fermentation

Tests API 32C System: With the use of API 32C system, sugar fermentation test was carried out.

Isolates were first purified by sub culturing on Sabouraud dextrose agar at least 2 to 3 times. Intermittent staining was done with Lacto phenol cotton blue and viewed under the X100 objective lens to be sure the isolate was yeast. A suspension from the culture medium was made in Sabouraud dextrose broth with a turbidity equivalent to 2 McFarland. From each of the suspension, 250µl was then transferred into an ampoule of the API 32C System medium. A volume of 135µl of prepared yeast basal medium suspension was then pipetted into wells containing 32 different freeze-dried sugars and incubated at 30°C for 24 to 48 hours. After incubation, growth was seen as turbidity in the well containing the sugars (substrate) in each cupule and was read using the ATBTM ExpressionTM or mini API instrument, or visually. Identification was obtained using the identification software into which the positive substrate code combinations are imputed and printed out. Software identification was done at the Chemistry Department at FIIRO (Federal Institute of Industrial Research, Oshodi) Lagos state.

2.4.6 Growth at 45°Cl

Suspected fungi colonies were inoculated into Sabouraud dextrose broth and incubated at 45°C and has been monitored for turbidity at different days for 7 to 10days. The ability of *C. dubilinensis* to grow at 45°C helps to differentiate it from *C. albicans*.

2.4.7 Chlamydiospore formation

Suspected culture isolates were inoculated onto Sabouraud dextrose agar plates using the Dalmau technique i.e., on a microscopic glass slide and a light-tomoderate inoculum of the greisen tube test was transferred by loop and streaked lightly over an area slightly greater than that to be covered by the subsequently placed, sterile, 22mm cover glass which was then incubated at 37°C for up to 3hours. Periodically, this begins between 1- 1.5hours. Cultures were observed through the cover glass for germ tube production. The low-power objective was used to scan the inoculated area; the high-dry objective was used to confirm the presence of germ tubes. After germ tube detection of after 3hours at 37°C, the plates were incubated at room temperature (18°C – 25°C) for chlamydiospore formation, a drop of Lacto phenol cotton blue was added to improve clarity and staining of chlamydiospore.

2.5 Statistical Analysis

Descriptive statistics was used to describe the study characteristics of the participants. Continuous variables were summarized as means, standard deviations (SD) for normally distributed or medians, interquartile ranges [IQR] in the case of skewed data and categorical variables were presented as proportions. The validated data was then transferred into SPSS version 20.0 for analysis. Data was summarized as number and percentages. The percentages of the different types of Candida species generated from the result of the study were compared between different disease conditions using Chi-square and ANOVA test. Statistical outcomes with *P*-value < 0.05 was considered to be significant.

3. RESULTS

Of the 250 participants involved in this study one hundred and eight (43.2%) were males and 142(57.8%) were females (Table 1). Overall prevalence of 12.8% (32/250) of pure fungal found by culture method. The rate was higher in female 17.5% compared to 6.5% in their male counterparts. A peaked prevalence of 28.6% was found among age group 83-92 years compared to the least rate of 6.7% among 23-32 years age bracket. Eleven (34.4%) of the these isolates formed germ tubes as indicated in Fig. 1 showing apple green colonies on chromogenic medium showing *C. albicans*/*C. dublininesis*. On the other hand, 21(66.6%) could not form germ tube thereby confirming the presence of other Candida species. Also, all the 11 isolates that produced germ tube showed turbidity (growth) when cultured in the Sabouraud dextrose broth at 45^0C for 4-5 days which confirmed *C. albicans.* The prevalence rates of detection of other isolates after testing with Chrom TM candida agar and growth at 45^0C were 11 (34.4% for *Candida albicans*), 2(6.3% for *C. glabrata*), 6(18.8% for *C. parasilopsis*), 8(25.0% for *C. tropicalis*), 2(6.3% respectively for *C. krusei* and *C. kefry*) and finally 1(3.1% for *C. hellenica*). Fermentation tests were done on all the 32 Candida isolates and all fermented different sugars thus helped in their identifications (Vides 1-3).

3.1 Specimens Yielding Pathogens

Of the total urine samples collected and analyzed for this study, 81 (32.4%) pure fungal and bacterial growths were isolated. Also, 78 (31.2%) cultures from either fungi or bacteria did not

produce any growth while 65 (26.0%) others did not produce any significant growth including 26 (10.4%) with mixed growth. Of the 81 bacteria isolated, only 49 (60.5%) of them had pure growth were bacteria while the rest 32(39.5%) were fungi. The bacteria isolates were *Escherichia coli* 25(51%), *Klebsiella aerogenes* 7(14.3%), *Staphylococcus aureus* 2(4.1%), *Pseudomonas aeroginosa* 7(14.3%), and *Staphylococcus saprophyticus* 8 (16.3%). Of the 32 fungal isolates, 7(21.9%) were from the urine specimens of the male participants while 25(79.1%) were from female participants. Among the 10 samples collected from male participants aged 13-22 years, only 1(10.0%) and 2 (16.7%) out of 12 samples from their female counterparts with same age group all produced fungal growth. On the other hands, samples tested from 21 male participants in age group 23-32 years did not yield (0.0%) but 3(12.5%) from 24 female samples in the same age group produced pure fungal growths. However in age group 33-42 years with 17 male samples, one (5.9%) specimen as well as 6 (15.0%) of the 40 female produced fungal growth. Furthermore, in age range 83-92, 1(16.7%) sample from 6 collected from the male participants and 1(100%) female specimen all produced fungal growth as indicated in Vides 1-3 and Table 2).

3.2 Age Distribution of Participants Infected with Candida Species

A total of 11 *Candida albicans* were isolated in all the age groups, the highest numbers of the isolates 3(27.3%) were found within the age group 43-52 years. For *C. glabrata,* only 1(50.0%) isolate each was found in the age groups 13-22 years and 33-42 years. One (50.0%) isolate of *C. krusei* was identified each from the age groups 63-72 years and 83-92 years and in the age groups 23-32 years and 33-42 years, one (50.0%) isolate of *C. kefry* was also identified. *C. parasilopsis* had a total of 6 (18.8%) isolates; the highest number 3(37.5%) was recorded in the age group 33-42 years. As regards *C. hellenica,* only one (50.0%) isolate was found, in the age group of 23-32 years (Vide 2; Table 2 and Fig. 2).

3.3 *In vitro* Antifungal Susceptibility Pattern of Candida Isolates

All the 32 Candida isolates were tested against 6 different antifungal discs; Ketoconazole, Fluconazole, Nystatin, Itraconazole, Terbinafine and Griseofulvin. Seven of the 11 *Candida albicans* isolated were mostly susceptible to Ketoconazole and 6 of the *Candida albicans* isolated were mostly susceptible Fluconazole. Two were moderately sensitive to Nystatin and Itraconazole while all the 11 isolates were resistant to Griseofulvin. The 2 *Candida glabrata* isolated were susceptible to Ketoconazole and moderately sensitive to Nystatin. Only one each of the isolates was susceptible to fluconazole, Itraconazole and Terbinafine. Four isolates of *C. parasilopsis* were susceptible to Ketoconazole and 5 of the isolates were susceptible to Fluconazole. Three each of the isolates were moderately sensitive to Nystatin and Terbinafine. One of the two, *C. krusei* was more susceptible to Nystatin, Ketoconazole, Fluconazole and moderately sensitive to Itraconazole. Similarly, only one of the 2 *C. kefyr* isolated was susceptible to Itraconazole. *C. hellenica* was only susceptible to Nystatin while all the fungal isolates were resistant to Griseofulvin (Vide 3; Table 3).

Table 1. Preponderance distribution of fungal UTI by age and sex

Age group (years)	No of male participants tested	No (%) of male participants with fungal infection	No of female participants tested	No (%) of female participants with fungal infection	Total No of participants tested	Total No (%) of participants with fungal infection
13-22	10	1(10.0)	12	2(16.7)	22	3(13.6)
23-32	21	0(0.0)	24	3(12.5)	45	3(6.7)
33-42	17	1(5.9)	40	6(15.0)	57	7(12.3)
43-52	10	2(20.0)	22	3(13.6)	32	5(15.6)
53-62	12	2(16.7)	20	3(15.0)	32	5(15.6)
63-72	23	0(0.0)	12	5(41.7)	35	5(14.3)
73-82	9	0(0.0)	11	2(18.2)	20	2(10.0)
83-92	6	1(16.7)	1	1(100.0)	7	2(28.6)
Total	108	7(6.5)	142	25(17.6)	250	32(12.8)

Chi square 19.8, d.f 6; Probability 0.003

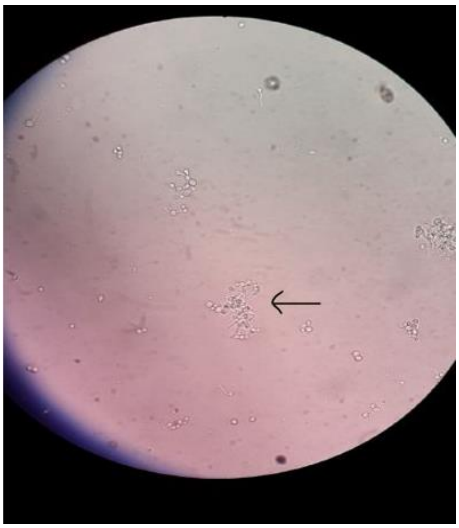


Fig. 1. Germ Tube Indicating *Candida Albicans*

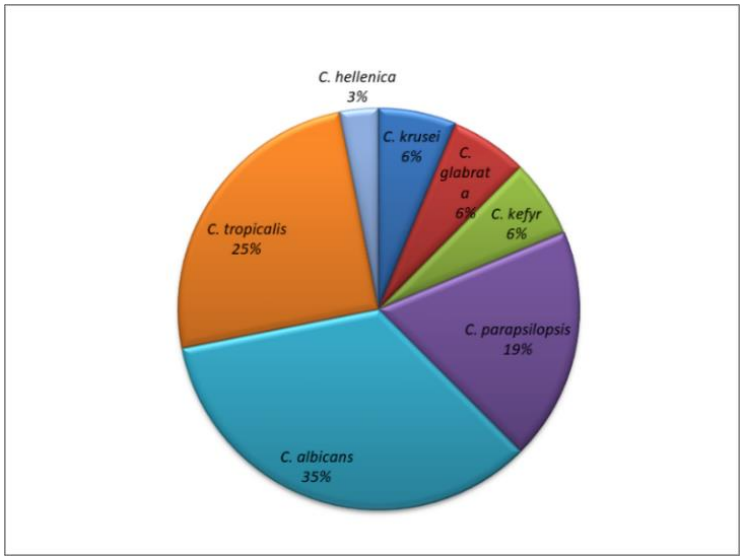


Fig. 2. Distribution of 11 Candida Species Isolated from Participants

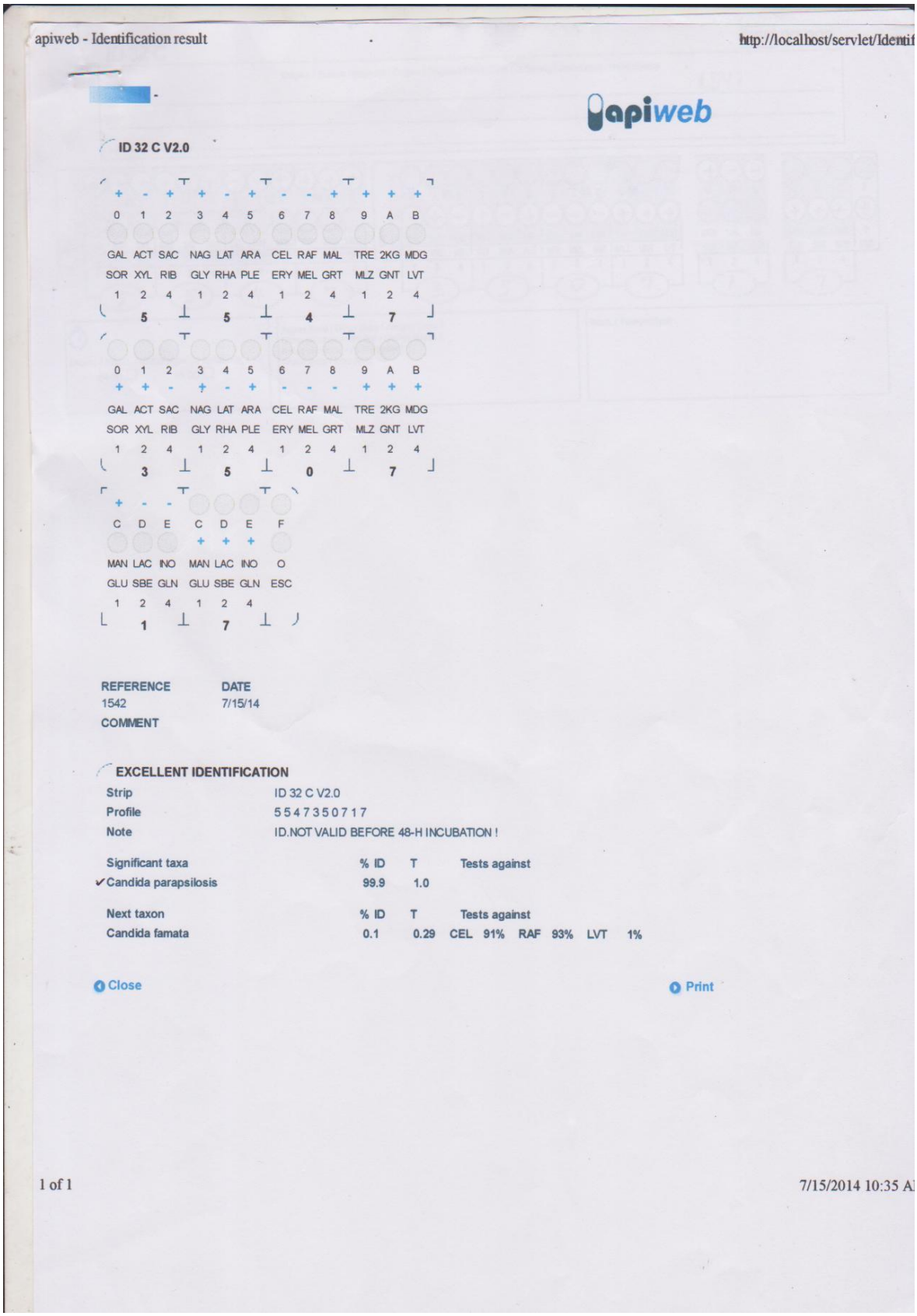
Table 2. Percentage distribution of candida species by age group

Age Groups (Years)	Organisms Isolated(n=32)						
	C. albican (%)	***C. glabrata*** (%)	***C. parapsilopsis*** (%)	***C. tropicalis*** (%)	***C. krusei*** (%)	***C. kefyr*** (%)	***C.hellenica*** (%)
13-22	1(9.1)	1(50.0)	0(0.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)
23-32	1(9.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	1(100.0)
33-42	1(9.1)	1(50.0)	3(50.0)	1(12.5)	0(0.0)	1(50.0)	0(0.0)
43-52	3(27.3)	0(0.0)	1(16.7)	1(12.5)	0(0.0)	0(0.0)	0(0.0)
53-62	1(9.1)	0(0.0)	1(16.7)	3(37.5)	0(0.0)	0(0.0)	0(0.0)
63-72	2(18.2)	0(0.0)	1(16.7)	1(12.5)	1(50.0)	0(0.0)	0(0.0)
73-82	1(9.1)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	0(0.0)	0(0.0)
83-92	1(9.1)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)
Total	11(34.4)	2(6.3)	6(18.8)	8(25.0)	2(6.3)	2(6.3)	1(3.1)

Table 3. *In vitro* antimicrobial susceptibility pattern

Candida species	Nystatin			Ketocunazole			Fluconazole			Itraconazole			Griseofulvin			Terbinafine		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans(11)	2	6	3	7	2	2	6	2	3	2	5	3	0	0	11	1	4	6
C.glabrata(2)	0	2	0	2	0	0	1	0	1	1	1	0	0	0	2	1	0	1
C. parapsilopsis(6)	2	3	1	4	0	2	5	0	1	2	1	3	0	0	6	0	3	3
C. krusei(2)	1	0	1	1	0	1	1	1	0	0	2	0	0	0	2	0	0	2
C.kefyr(2)	0	2	0	0	0	2	1	0	1	1	0	1	0	0	2	0	0	2
C.tropicalis(8)	2	4	2	5	2	1	2	3	3	2	3	3	0	0	8	2	2	4
C.hellenica(1)	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1

Key: S-Sensitivity; I- Intermediate; R- Resistance



apiweb - Identification result

http://localhost/servlet/Identif

apiweb

ID 32 C V2.0

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GAL	ACT	SAC	NAG	LAT	ARA	CEL	RAF	MAL	TRE	2KG	MDG
SOR	XYL	RIB	GLY	RHA	PLE	ERY	MEL	GRT	MLZ	GNT	LVT
1	2	4	1	2	4	1	2	4	1	2	4
	5			5			4			7	

0	1	2	3	4	5	6	7	8	9	A	B
+	+	-	+	-	+	-	-	-	+	+	+
GAL	ACT	SAC	NAG	LAT	ARA	CEL	RAF	MAL	TRE	2KG	MDG
SOR	XYL	RIB	GLY	RHA	PLE	ERY	MEL	GRT	MLZ	GNT	LVT
1	2	4	1	2	4	1	2	4	1	2	4
	3			5			0			7	

C	D	E	C	D	E	F
+	-	-	+	+	+	
MAN	LAC	INO	MAN	LAC	INO	O
GLU	SBE	GLN	GLU	SBE	GLN	ESC
1	2	4	1	2	4	
	1			7		

REFERENCE 1542

DATE 7/15/14

COMMENT

EXCELLENT IDENTIFICATION

Strip ID 32 C V2.0

Profile 5 5 4 7 3 5 0 7 1 7

Note ID.NOT VALID BEFORE 48-H INCUBATION !

Significant taxa	% ID	T	Tests against
✔Candida parapsilosis	99.9	1.0	

Next taxon	% ID	T	Tests against					
Candida famata	0.1	0.29	CEL	91%	RAF	93%	LVT	1%

Close

Print

1 of 1

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Fig. 3. Identification Results (Vide 1)

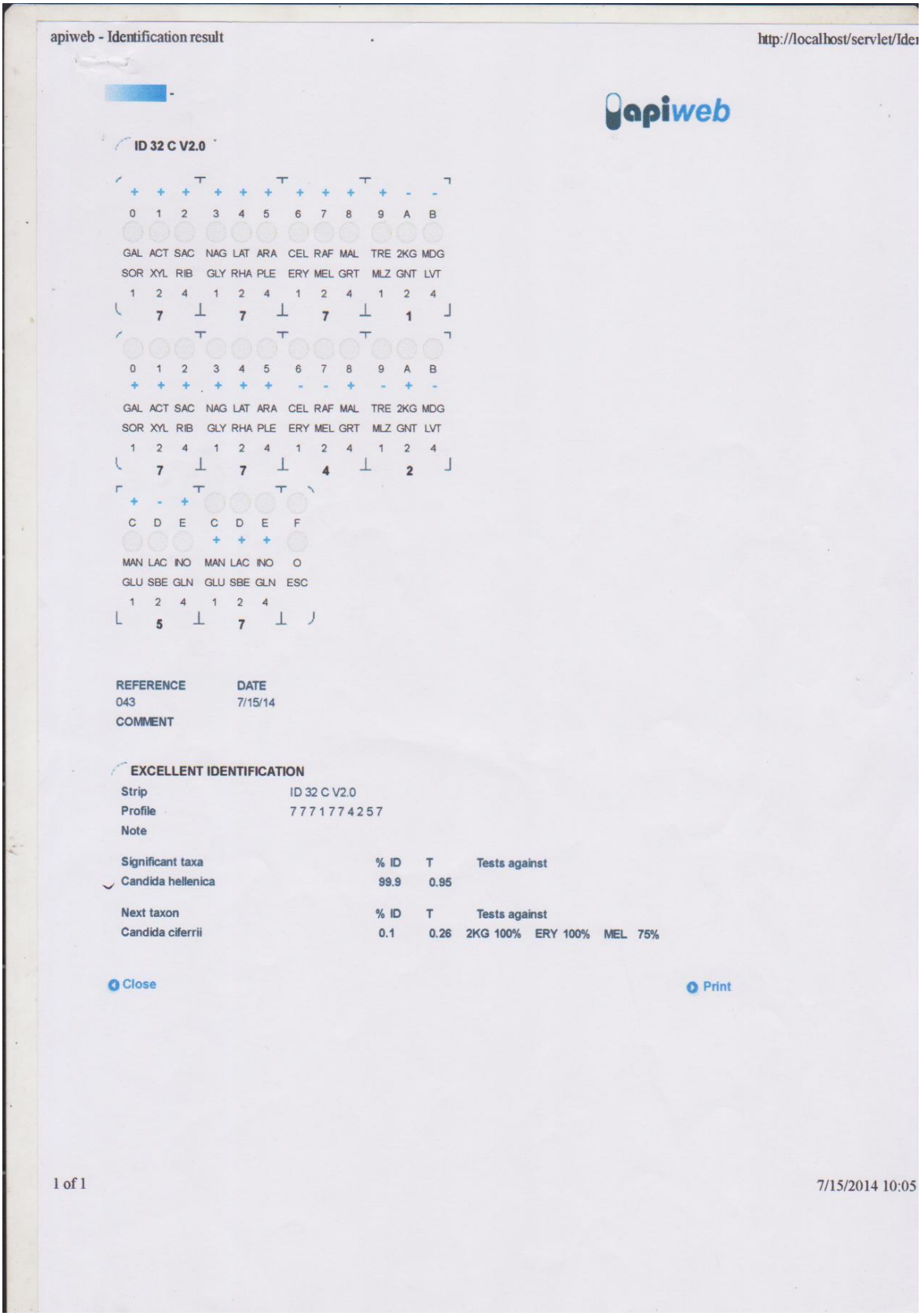
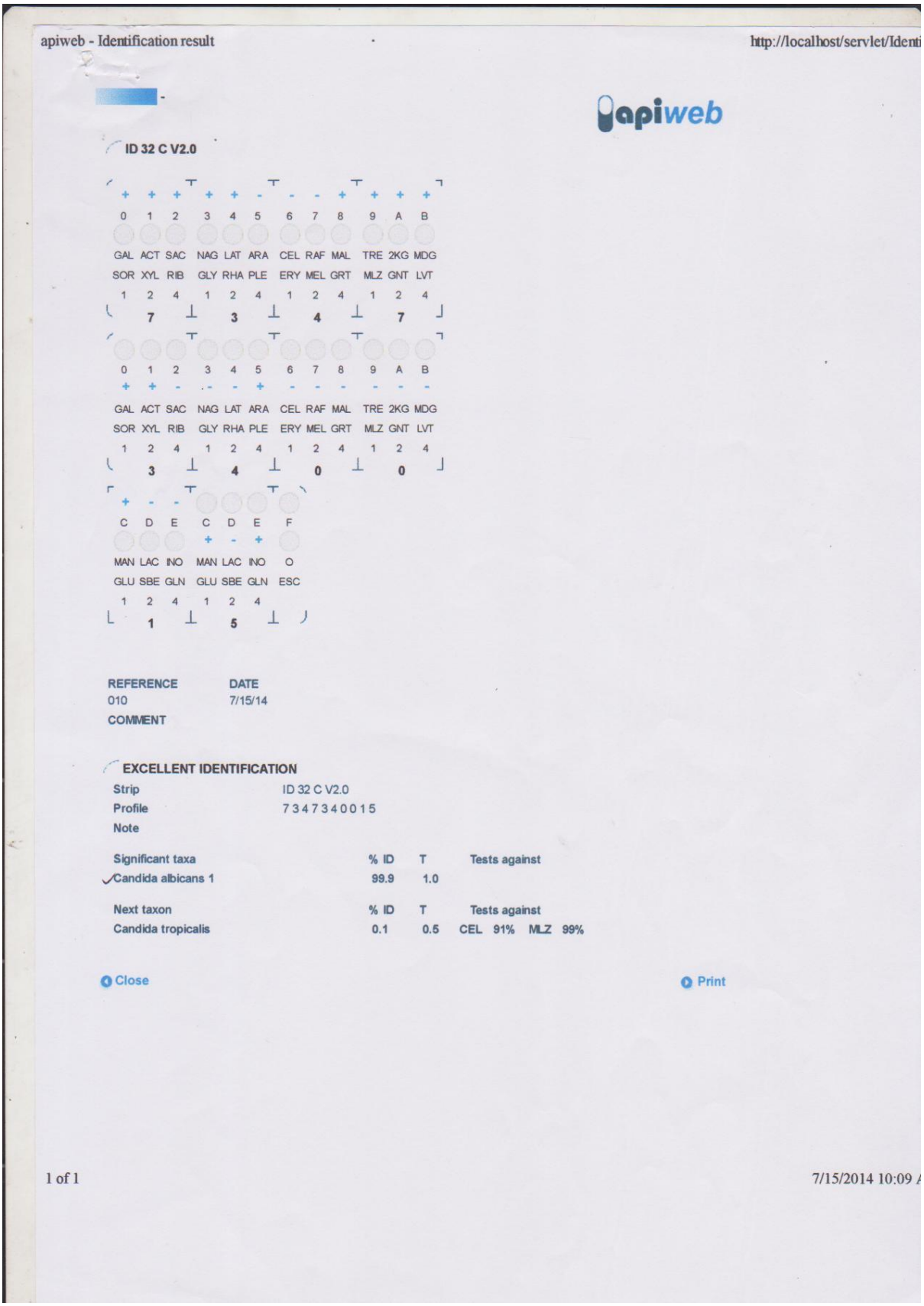


Fig. 4. Identification Results (Vide 2)


apiweb - Identification result

http://localhost/servlet/Identi

apiweb

ID 32 C V2.0

0	1	2	3	4	5	6	7	8	9	A	B
+	+	+	+	+	-	-	-	+	+	+	+
GAL	ACT	SAC	NAG	LAT	ARA	CEL	RAF	MAL	TRE	2KG	MDG
SOR	XYL	RIB	GLY	RHA	PLE	ERY	MEL	GRT	MLZ	GNT	LVT
1	2	4	1	2	4	1	2	4	1	2	4
	7			3			4			7	

0	1	2	3	4	5	6	7	8	9	A	B
+	+	-	-	-	+	-	-	-	-	-	-
GAL	ACT	SAC	NAG	LAT	ARA	CEL	RAF	MAL	TRE	2KG	MDG
SOR	XYL	RIB	GLY	RHA	PLE	ERY	MEL	GRT	MLZ	GNT	LVT
1	2	4	1	2	4	1	2	4	1	2	4
	3			4			0			0	

C	D	E	C	D	E	F
+	-	-	+	-	+	
MAN	LAC	INO	MAN	LAC	INO	O
GLU	SBE	GLN	GLU	SBE	GLN	ESC
1	2	4	1	2	4	
	1			5		

REFERENCE 010 **DATE** 7/15/14

COMMENT

EXCELLENT IDENTIFICATION

Strip ID 32 C V2.0

Profile 7347340015

Note

Significant taxa	% ID	T	Tests against
✓Candida albicans 1	99.9	1.0	

Next taxon	% ID	T	Tests against
Candida tropicalis	0.1	0.5	CEL 91% MLZ 99%

Close

Print

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Fig. 5. Identification Results (Vide 3)

4. DISCUSSION

This study reported an overall rate (12.8%) for candiduria among individuals accessing care at Lagos State University Teaching Hospital, Nigeria. This rate was lower than 16.5% candiduria found among similar population admitted in Golestan and Emam Khomeini hospitals of Ahvaz, Iran [9]. An incidence rate of 12.8% of Candida species were reported in this study which correlates with the findings of Richards *et al.* [19] and in contrast with the report of Sehgal, [25] which reported an incidence value of 54% among patients in Northern Nigeria. Our study detected 11(34.4%) for *Candida albicans* and other isolates such as 2(6.3% for *C. glabrata*), 6(18.8% for *C. parasilopsis*), 8(25.0% for *C. tropicalis*), 2(6.3% for *C. krusei* and *C. kefry*) and 1(3.1% for *C. hellenica*). This study revealed a greater proportion of Candida Non Albicans (CAN) isolates from urine samples, which points to the emergence of strains which are treatment resistant in the study participants. The presence of candiduria in critically ill patients has been regarded as an indicator of invasive candidiasis [5]. However, the high preponderance of CNA as a cause of candidiasis in hospital settings has been observed globally at any site of infection, including the urinary tract [9,26,27] . The importance of this fact is that CNA species are likely to be resistant to antifungal agents, which calls for urgent need to identify various *Candida* species, as well as to assess their susceptibility profile to antifungal agents in hospitalized patients [16,28,29] .

In this study, *C. albicans*, *C. tropicalis*, and *C. parapsillosis* were the main isolates. The prevalence rates of *Candida* species detected in our study when compared with other previous studies [3,20,30] regarding candiduria shows that the predominant type is *C. albicans* among the seven candida species [3] taking 2nd position and *C. tropicalis* in three studies [3,18,31], with the same trend found in our study. A study reported that *C. tropicalis* was most prevalent [20]. The rate of 4.7% for *C. parapsilosis* found in this study, falls within the range 0 to 17.4% [3,20,30] reported from previous studies. There was a relationship in the preponderances of *Candida* species from reported studies [3,10,20] when studies from different regions were evaluated and compared with our own [18,20,32]. *C. albicans* is the most frequently found species in the digestive tracts of healthy people, and it has greater pathogenic mechanisms when compared to other *Candida* spp. [20]. The emergent *C. tropicalis* may lead to UTI in patients which present with chronic degenerative disorders [29,33].

By gender, higher rate of candiduria was detected in women [34] 17.5% among females to 6.5% for males. Previous studies have demonstrated that as high as 30% of asymptomatic women may experience persistent vulvovaginal colonization by *Candida* spp. The female body conformation which align with the bladder and kidneys can aid or prompt urinary tract infections [22,35] . The peak rate of 28.6% was found in age bracket 83-92 years and lowest rate 6.7% detected among age range 23-32 years. This agrees with previous reports which affirm that most of the study subjects are of age indicating natural modifications of their dysfunctional immune system, leading to their long hospital stay in critical care units requiring urinary system supported devices [6,27]. Furthermore, of the thirty-two fungal isolates, 7(21.9%) were from the urine specimens of the male participants while 25(79.1%) were from female participants.

The prevalence of *C. glabrata* in our study (6.3%) falls within the range of 0 to 12.5 % found in other earlier studies;[20,30,31]. The present finding supports previous reports, which demonstrates that *C. glabrata* has emerged in tertiary hospitals in recent years, both in Nigeria and in other countries [36,37]. The *C. parapsilosis* complex is noted as a primary agent of urinary tract infections in very sick patients under admission due to its capacity to form biofilm [38,39]. Not all categories of the *C. parapsilosis* complex are virulent; among them, *C. metapsilosis* appears to present the lowest virulence, but this evidence is still limited [40] . This calls for differentiating *C. parapsilosis* in clinical studies, including isolates from other anatomic sites [41]. In this study, the six (18.8%) isolates that belong to the *C. parapsilosis* complex were identified as *C. parapsilosis (sensu stricto)*.

Many reports have demonstrated the emergence of antifungal resistance, especially Fluconazole (FLC) resistance in *C. albicans* (Odds, 1988 [42], however, all of the *C. albicans* isolates were susceptible to Nystatin, Ketocunazole, Griseofulvin and Itraconazole. Moreover, the non-albicans species were also susceptible to the tested drugs, except *C. krusei*, which is intrinsically resistant to FLC. Furthermore, all isolates of *C. glabrata* were also resistant. Resistance to FLC or Nyst has already been

reported in *C. glabrata* [5] and *C. tropicalis* [29]. Among the 11 *Candida albicans* detected 7(63.6%) were mostly Ketoconazole susceptible while 6 of the *Candida albicans* also detected were Fluconazole susceptible. Two were moderately sensitive to Nystatin and Itraconazole while all the eleven isolates were resistant to Griseofulvin. The two *Candida glabrata* isolated were susceptible to Ketoconazole and moderately sensitive to Nystatin. In South America, some findings from different regions evaluated the susceptibility to FLC of *Candida* species isolated from urine [10,43,44]. Adopting the broth microdilution method to evaluate *C. albicans* isolates, one study showed no resistance to FLC [32], as we have found, and another showed 15.0% of resistance 5 thereby corroborating our findings. Applying the disk diffusion method, high rates of FLC resistance were observed [3]. With reference to *C. parapsilosis*, FLC resistance [32] was not reported.

5. CONCLUSION

This study reported a variety of Candida species among the study participants associated with urinary system indicating high degree of public health significance. Early and accurate species detection, including anti-microbial susceptibility and characterization of isolates, is desirable for evaluation of appropriate therapies for the management of recurrent candiduria and other related fungal infections. The Candida non albicans also cause damages to the kidney and their effects if not handled as early as possible may be more disastrous than the *Candida albicans*. The apparently healthy participants with evidence of candida in their urine must be adequately investigated with restraints before making a pronouncement [16]. Therefore, the surveillance and effective efficient control measures should be put in place to monitor the trend of the circulation of these pathogens ravaging our communities.

ETHICAL APPROVAL AND CONSENT

Ethical approval was sought and approved before the study started by the LASUTH institutional review board (Reg. No. NHREC04/04/2018) while written consent was obtained in writing from the participants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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