



## Evaluation of Antifungal Susceptibility Profile of *Candida* Species Isolated from Female Patients Attending Aminu Kano Teaching Hospital (AKTH)

\*<sup>1</sup>Taura, D. W.    <sup>1</sup>Yakubu, G.    <sup>2</sup>Panda, T. W.    <sup>3</sup>Dagona, A.G.

<sup>1</sup> Department of Microbiology, Bayero University, P.M.B.3011, Kano- Nigeria.

<sup>2</sup> Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano- Nigeria.

<sup>3</sup> Department of Biological Sciences, Faculty of Science, Federal University, Gashua- Yobe State

### ABSTRACT

The increasing incidence of *Candidiasis* affecting the genitourinary tracts as well as the introduction of new antifungal drugs has recently encouraged the need for performing fungal susceptibility tests. The study was aimed at evaluating the antifungal susceptibility profile of *Candida* species isolated from female patients attending Aminu Kano Teaching Hospital (AKTH), with suspected Candidal infections between August, 2012 to June, 2013. Five hundred and twenty one (521) clinical samples comprising 342 urine and 179 high vaginal swabs were cultured on Sabouraud dextrose agar. The *Candida* species isolated were identified to species level using Chromogenic agar and API 20 C AUX test kit. Antifungal susceptibility tests were performed using commercially prepared single antifungal disc (Bioanalyse Turkey). Out of these 521 samples analyzed only 59 yielded *Candida* species giving the overall prevalence of 11.3% with *Candida albicans* 22 (37.3%) as the common species isolated followed by *C. glabrata* 19 (32.2%), *C. tropicalis* 5(8.5%), *C. krusei* 3 (5.1%), *C. magnoliae* 3 (5.1%), *C. lusitaniae* 2 (3.4%), *C. parapsilosis* 2 (3.4%), *C. famata* 2 (3.4%) and *C. guilliermondii* 1 (1.7%). The antifungal susceptibility test shows that 81.4% of the isolates were susceptible to ketoconazole and only 3.4% to nystatin. However, 33.9% were susceptible, 13.6% intermediate susceptible and 52.5% resistant to fluconazole. Similarly 28.8% were susceptible, 5.1% intermediate susceptible and 66.1% resistant to voriconazole. Additionally 25.4% were susceptible, 11.9% intermediate susceptible and

62.7% resistant to flucytosine. All the *C. krusei* isolates were completely resistant to azole drugs while *C. famata* were resistant to all the drugs tested. Two quality control strains of *Candida* namely: *Candida tropicalis* ATCC 750 and *Candida albicans* ATCC 90028 were used.

**Keywords:** Evaluation, Antifungal susceptibility Profile, *Candida* species

### INTRODUCTION

*Candida* species are opportunistic yeast affecting the genitourinary tracts. *Candida* is listed by the centre for disease control (CDC) as a cause of sexually transmitted disease (Prescott *et al.*, 2008). No other mycotic pathogen produces as diverse a spectrum of opportunistic disease in humans as does *Candida*. *Candida* species are important nosocomial pathogens and can be transmitted sexually (Taffeng *et al.*, 2004). *Candida* species are the normal micro biota within the gastrointestinal tracts, respiratory tracts, vaginal area and the mouth (Prescott *et al.*, 2008). *Candidiasis* refers to a range of infection caused by species of fungal genus *Candida*. The infections can be acute or chronic, localized or systemic. Disseminated *Candidiasis* is frequently life threatening. The great majority of these infections are caused by *Candida albicans* (Greenwood *et al.*, 1992). The incidence of genitourinary tract infection is much higher in females during adolescence and childbearing years (Sobel, 2004). *Candida* is found in the vagina of 35 - 50% of

healthy women. Under some conditions, such as reduced immunity, prolonged antibiotics therapy, use of contraceptives, malnutrition, pregnancy, diabetes, obesity, tissue transplant, use of immunosuppression drugs (Corticosteroids), neutropenia, *Candida* may become pathogenic and cause Candidiasis (Okungbowa *et al.*, 2003). *Candida* species are the second most frequent isolates from blood cultures in hospitals with large populations of immunocompromised patients (Beck Sague *et al.*, 1993 Tاتفeng *et al.*, 2004). In broader sense fungal diseases became recognized as being of clinical importance in the second half of the last century. This is mainly due to advancement in medical technologies (Rizvi *et al.*, 2011). With the remarkable modern advances in medicine, there has been an increase in the number of immuno compromised individuals who need extensive care in the hospitals (Rizvi *et al.*, 2011). This has resulted in a rise in the incidence of fungal infections, especially those due to *Candida species* (Rizvi *et al.*, 2011). Among the species only nine are frequent pathogens for human (Andy *et al.*, 2004). *Candida albicans* is the most common fungal causative agent in superficial and deep seated Candidiasis. However, non- albicans are also being implicated in recent years (Gullo, 2009). However, infection of the vulva and vagina with *Candida albicans* is termed vulvovaginal Candidiasis. This is characterized by intense vulval and vaginal irritation (itching) which often gives rise to inflammation, soreness, redness and white spots in and around the genital tract. Also, painful urination and intercourse are associated with the infection. In about 5% cases of Candidal vulvovaginitis, the disease has a chronic course showing frequent and refractory episodes (Ferrer, 2000). Additionally, various studies have shown that nearly 75% of all women will experience at least one attack of Candidal vulvovaginitis during their life time and suffer multiple episodes (Ferrer, 2000; Saporiti *et al.*, 2001). The presence of *Candida* in urine is called candiduria. This is rarely encountered in otherwise healthy people with structurally normal urinary tract (Schonebeck and Ansehn, 1972; Kauffman, 2005 and Bukhary, 2008). It is however of common occurrence in hospitalized patients. *Candida species* account for almost 10-15% of nosocomial urinary tract infections (UTIs) (Kauffman *et al.*, 2000; Lundstrom and Sobel, 2001 and Kauffman, 2005). In the United States it is estimated that the incidence of candiduria was 25,000 cases per year. And approximately one third of hospitalized patients with urine cultures yielding

*Candida* were in the Intensive care unit (ICU) where bladder catheter use was high (Shay and Miller, 2004). The aim of the present research was to carry out antifungal potency evaluation test against *Candida species* isolated from female patients attending Aminu Kano Teaching Hospital. These antifungal agents inhibit macromolecule synthesis (Flucytosine), impair membrane barrier function (polyenes), inhibit ergosterol synthesis (allylamines, thiocarbamates, azole derivatives, and morpholines) or interact with microtubules (Vanden *et al.*, 1997).. Emergence of drug resistance among yeast isolates and consequent increase in serious fungal infections have been reported (DeMuri *et al.*, 1995). The mechanism of resistance to these antifungal agents by yeast isolates are purely chromosomal as *Candida* species lack plasmid or other natural mechanism capable of transferring genetic materials between strains (Odds *et al.*, 2003). Knowledge of mechanisms of antifungal resistance has been valuable in identifying resistant isolates and using them to validate in vitro measurement systems (Ghannoum, *et al.*, 1999, Moore *et al.*, 2000, Vanden Bossche, *et al.* 1998 and White *et al.*, 1998).

### Study Area

The study was conducted in the microbiology laboratory of Aminu Kano Teaching Hospital (AKTH) Kano. The hospital is located in the metropolitan city of Kano in Tarauni LGA. It serves as a referral centre for diseases that could not be manage by other hospitals within the Kano environs and some neighbouring states.

### Study Population

The study targeted only female patients that were sent to microbiology laboratory by clinicians with a request form and are suspected having Candidiasis. Those that did not meet the above criteria were not included in this study.

### Sample Size

A total of 521 clinical samples including 342 urine and 179 high vaginal swabs were collected and analyzed from female patients attending Aminu Kano Teaching Hospital with suspected cases of Candidiasis between august, 2013 to June, 2014.

### Ethical Clearance

This was obtained from the hospital ethical committee prior to the commencement of the study. In addition, an informed consent was sought from patients before

their enrolment. Those that refused to be recruited after much explanation were not considered in the research.

### SAMPLE COLLECTION

**Urine Collection and Processing** Fresh morning mid-stream urine samples were aseptically collected in clean, dry culture bottles. Patients have been told to first allow urine to flow out and collect the mid-stream in a sterile screw cap container and then replace the lid firmly and bring the samples to the microbiology laboratory. For each specimen collected, Age, Sex, Laboratory number, and Name of patients were clearly labelled. At laboratory, the urine was first transferred into a sterile test tube and centrifuged at 3000 rpm for 5 minutes. After centrifugation, the supernatant was decanted and the sediment was streaked on to SDA (Himedia) with the aid of a standard wire loop calibrated to hold (0.01) ml urine. Specimens were incubated at 37<sup>0C</sup> for 24- 48 hrs. Wet film was then prepared and the remaining portion of the sediment was placed on a dried grease free slide and stained using Gram's method. Microscopy was followed to observe the presence of yeast cells.

**High Vaginal Swab Collection and Processing** High vaginal swab samples were collected aseptically in sterile cotton tipped swab stick by a trained female nurse officer. The specimens were properly labelled and then transported to the laboratory for analysis. Upon receipt at the laboratory samples were inoculated on SDA (Himedia) directly using the cotton tipped swab stick and then spread by streaking with standard sterile wire loop. Specimens were incubated at 37<sup>0C</sup> for 24 - 48 hrs. The swab was then replaced back to its container and 2-3 drops of normal saline were added. This was shaken vigorously and dispensed in to a sterile test tube. The specimen was centrifuged at 3000 rpm for 5 minutes, supernatant was decanted and a wet film prepared to observe the presence of yeast cells. Part of the sediment was used for Gram staining. The stained slides were examined under microscope for budding yeast cells. Identification and Characterization Yeast isolates were identified to specie level using Chromogenic Agar and API 20C Aux kit Biomeriuxu company of France. Inoculation on Chromogenic Agar Yeast isolates were first sub cultured on SDA (Himedia) to obtained pure/ fresh colonies of the organism to be tested. Chromogenic agar was then prepared according to manufacturer guidelines. The yeast isolates were sub culture on to the media and incubated for 24 - 48 hrs. API 20 AUX C Kit, Yeast

isolates were first sub culture on SDA in order to obtain fresh colonies of the organism. Colonies were picked by successive touches and introduced in to a sterile test tube containing 2ml (0.85%) normal saline. The turbidity was measured visually to match with 2 Mc Farland standard as directed by the manufacturer. And then 100 µl of the suspension was pipetted with micro titre pipette and dispensed into API media. Further transfer of suspension was done from API media to API strip that is made up of 20 cupules. The cupules were filled with the API media already containing the test organism and then incubated at 29<sup>0C</sup> for 48 – 72 hrs as directed by the manufacturer. The cupules are already pre-coated with sugars except the first cupule that serve as control. After incubation, growth in each cupule was compared with the control. A cupule that is more turbid than the control was recorded in the result sheet as positive. On the result sheet the tests are separated into seven groups with each group bearing three digits numbers of 1, 2 and 4. Therefore, by adding the numbers corresponding to positive reactions within each, a 7 digits number is obtained which constitute the numerical profile. The final identification was done by checking the seven digits number in the API booklet, where yeast species are provided with codes that correspond to Excellent, Very Good, Good and Acceptable Identifications. Antifungal Susceptibility Test Disk diffusion method using commercially prepared antifungal single disk (Bioanalyse) Turkey was employed as recommended by (National committee for clinical laboratory standards (NCCLS-M44A, 2004).

*Procedure:* Fresh culture of each isolate of not more than 18- 24 hrs old as recommended by the company were suspended in a sterile test tube containing 5ml (0.85%) normal saline. The turbidity was measured visually to match with 0.5 Mc Farland standard. Sterile cotton tipped swab stick was then dipped into the suspension containing the organism to be tested. The cotton tipped swab stick was then raised above the suspension and pressed along the inner wall of the test tube to remove excess moisture. This was inoculated on dried plate of Muller Hinton agar supplemented with 2% Glucose and 0.5g Methylene Blue by swabbing at 60<sup>0</sup> in three dimensions for uniformity as recommended by (National committee on clinical laboratory standards M44A, 2004). The glucose supports the growth of *Candida species* while Methylene Blue enhanced zone and edge clarity. Sterile single discs were aseptically placed on each inoculated plate with the aid of sterile forcep and



incubated at 37<sup>OC</sup> for 18 - 48 hrs. The zone of inhibition produced by each antifungal disc was measured with a calibrated ruler, and then interpreted based on the interpretive break points recommended by ((CLSI, M44, 2004) M44, 2004). In this case only fluconazole and voriconazole interpretive break points were provided. However, the response to other antifungal agents for which no interpretive break points provided by CLSI, these were interpreted according to the manufacturer's instructions (Table 1). Also, two quality control strains of *Candida* namely: *C. albicans* ATCC 90028 and *Candida tropicalis* ATCC 750 were used.

## RESULTS

In this research a total of 521 samples including 342 and 179 Urine and High vaginal swabs respectively were processed and analyzed, out of which only 59 (11.3%) were *Candida species* (Table 1). However, the prevalence of *Candida species* in urine was 8 (2.3%). The species isolated were *C. albicans* of about 3 (37.5%), *C. glabrata*, *C. tropicalis*, *C. famata*, *C. parapsilosis*, and *C. magnoliae* each with 1(12.5%) respectively. Higher prevalence of 51(28.5%) was recorded in high vaginal swab samples which includes *C. albicans* 19 (37.3%) followed by *C. glabrata* 18(35.3%), *C. tropicalis* 4(7.8%), *C. krusei* 3(5.9%), *C. magnoliae* and *C. lusitaniae* 2(3.9%) each, *C. parapsilosis*, *C. famata*, and *C. guilliermondii* 1(1.96%) respectively. The predominant specie isolated in both the clinical samples was *C. albicans* (Table 2). With the respect to antifungal susceptibility profile of urine isolates, the result shows that 75% of

the isolates were susceptible to Ketoconazole. While 62.5% of the isolates were susceptible to Fluconazole, only 50% were susceptible to Voriconazole and Flucytosine respectively. Interestingly, *C. famata* was resistant to all the agents and in addition none of the isolates was susceptible to Nystatin. Similarly *C. tropicalis* was only susceptible to ketoconazole while *C. albicans* was susceptible to all the agents except Nystatin (Table 3). However, high vaginal swab (HVS) isolates shows a different susceptibility pattern on comparison with urine counterpart in which 82.4% of the isolates were susceptible to Ketoconazole and were 29.4% susceptible, 13.73% intermediate susceptible and 56.9% resistant to fluconazole. Similarly 25.5% of the isolates were susceptible to Voriconazole, 5.9% intermediate and 68.63% resistant. Highest resistance was observed in nystatin (96.1%). In addition, *C. famata* and *C. guilliermondii* were resistant to all the agents except that *C. famata* was susceptible to only Ketoconazole. Table 5 presents the overall susceptibility profile of the isolates. The result demonstrated that 81.4% were susceptible to Ketoconazole. However, 33.9% were susceptible to Fluconazole, 13.6% intermediate and 52.5% resistance. Similarly, Flucytosine 25.4% were susceptible, 11.9% intermediate and 62.7% resistance. Surprisingly, 96.6% of the isolates were completely resistant to nystatin. While only *C. albicans* and *C. glabrata* were susceptible to Nystatin, *C. guilliermondii* *C. krusei* were completely resistant to all the agents except that *C. krusei* was intermediate susceptible to flucytosine.

**Table1. Prevalence of *Candida species* in the clinical samples**

Samples	Number processed	Number positive	Prevalence (%)
Urine	342	8	2.3
High vaginal swab	179	51	28.5
Total	521	59	11.3

**Table2. Prevalence and distributions of *Candida species* in the clinical samples**

S/n	Isolates	High vaginal swab	Urine	Total
1	<i>C. albicans</i>	19(37.30)	3(37.5)	22(37.3)
2	<i>C. glabrata</i>	18(35.30)	1(12.5)	19(32.2)
3	<i>C. tropicalis</i>	4(7.80)	1(12.5)	5(8.5)
4	<i>C. krusei</i>	3(5.90)	0(0)	3(5.1)
5	<i>C. magnolia</i>	2(3.90)	1(12.5)	3(5.1)
6	<i>C. lusitaniae</i>	2(3.90)	0(0)	2(3.4)
7	<i>C. parapsilosis</i>	1(1.96)	1(12.5)	2(3.4)
8	<i>C. famata</i>	1(1.96)	1(12.5)	2(3.4)
9	<i>C. guilliermondii</i>	1(1.96)	0(0)	1(1.7)
	Total	51	8	59(100)

Key = Values in parentheses are percentage, S/n serial number

**Table3. Antifungal susceptibility profile of urine *Candida* isolates**

S/n	ISOLATES	N	FLU	VOR	NYS	5FC	KTC
			S I R	S I R	S I R	S I R	S I R
1	<i>C. albicans</i>	3	2 0 1	2 0 1	0 - 3	2 0 1	2 - 1
2	<i>C. glabrata</i>	1	1 0 0	0 0 1	0 - 1	1 0 1	1 - 0
3	<i>C. tropicalis</i>	1	0 0 1	0 0 1	0 - 1	0 0 1	1 - 0
4	<i>C. parapsilosis</i>	1	1 0 0	1 0 0	0 - 1	0 0 1	1 - 0
5	<i>C. famata</i>	1	0 0 1	0 0 1	0 - 1	0 0 1	0 - 1
6	<i>C. magnolia</i>	1	1 0 0	1 0 0	0 - 1	1 0 0	1 - 0
	Total	8	5 0 3	4 0 4	0 - 8	4 0 4	6 - 2
	Percentage %		62.5 0 37.5	50 0 50	0 - 100	50 0 50	75 - 25

Keys = N, number, Flu, fluconazole, Vor, voriconazole, Nys, nystatin, 5fc, flucytosine and KTC, ketoconazole, S sensitive, I intermediate sensitive, R resistant, S/n serial number

**Table 4: Antifungal susceptibility profile of high vaginal swab *Candida* isolates**

S/n	ISOLATES	N	FLU			VOR			NYS			5FC			KTC		
			S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1	<i>C. albicans</i>	19	5	1	13	5	0	14	1	-	18	1	0	18	16	-	3
2	<i>C. glabrata</i>	18	8	4	6	5	3	10	1	-	17	9	5	4	17	-	1
3	<i>C. tropicalis</i>	4	1	0	3	1	0	3	0	-	4	0	1	3	4	-	0
4	<i>C. krusei</i>	3	0	0	3	0	0	3	0	-	3	0	1	2	0	-	3
5	<i>C. magnolia</i>	2	0	1	1	1	0	1	0	-	2	0	0	2	2	-	0
6	<i>C. lusitaniae</i>	2	0	1	1	0	0	2	0	-	2	0	0	2	1	-	1
7	<i>C. parapsilosis</i>	1	1	0	0	1	0	0	0	-	1	0	0	1	1	-	0
8	<i>C. famata</i>	1	0	0	1	0	0	1	0	-	1	0	0	1	1	-	0
9	<i>C. guilliermondii</i>	1	0	0	1	0	0	1	0	-	1	0	0	1	0	-	1
	<b>Total</b>	<b>51</b>	<b>15</b>	<b>7</b>	<b>29</b>	<b>13</b>	<b>3</b>	<b>35</b>	<b>2</b>	<b>-</b>	<b>49</b>	<b>10</b>	<b>7</b>	<b>34</b>	<b>42</b>	<b>-</b>	<b>9</b>
	<b>Percentage%</b>		<b>29.4</b>	<b>13.3</b>	<b>56.9</b>	<b>25.5</b>	<b>5.9</b>	<b>68.63</b>	<b>3.9</b>	<b>-</b>	<b>96.1</b>	<b>19.61</b>	<b>13.73</b>	<b>66.7</b>	<b>82.4</b>	<b>-</b>	<b>17.6</b>

Keys = N, number, Flu, fluconazole, Vor, voriconazole, Nys, nystatin, 5fc, flucytosine and KTC, ketoconazole, S sensitive, I intermediate sensitive, R resistant, S/n serial number

**Table 5: Overall susceptibility profile of the isolates**

S/n	ISOLATES	N	FLU			VOR			NYS			5FC			KTC		
			S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1	<i>C. albicans</i>	22	7	1	14	7	0	15	1	-	21	3	0	19	18	-	4
2	<i>C. glabrata</i>	19	9	4	6	5	3	11	1	-	18	10	5	4	18	-	1
3	<i>C. tropicalis</i>	5	1	0	4	1	0	4	0	-	5	0	1	4	5	-	0
4	<i>C. krusei</i>	3	0	0	3	0	0	3	0	-	3	0	1	2	0	-	3
5	<i>C. magnoliae</i>	3	1	1	1	2	0	1	0	-	3	1	0	2	3	-	0
6	<i>C. lusitaniae</i>	2	0	1	1	0	0	2	0	-	2	0	0	2	1	-	1
7	<i>C. parapsilosis</i>	2	2	0	0	2	0	0	0	-	2	0	0	2	2	-	1
8	<i>C. famata</i>	2	0	1	1	0	0	2	0	-	2	1	0	1	1	-	1
9	<i>C. guilliermondii</i>	1	0	0	1	0	0	1	0	-	1	0	0	1	0	-	1
	<b>Total</b>		<b>20</b>	<b>8</b>	<b>31</b>	<b>17</b>	<b>3</b>	<b>39</b>	<b>2</b>	<b>-</b>	<b>57</b>	<b>15</b>	<b>7</b>	<b>37</b>	<b>48</b>	<b>-</b>	<b>11</b>
	<b>Percentage %</b>		<b>33.9</b>	<b>13.6</b>	<b>52.5</b>	<b>28.8</b>	<b>5.1</b>	<b>66.1</b>	<b>3.4</b>	<b>-</b>	<b>96.6</b>	<b>25.4</b>	<b>11.9</b>	<b>62.7</b>	<b>81.4</b>	<b>-</b>	<b>18.6</b>

Keys = N, number, Flu, fluconazole, Vor, voriconazole, Nys, nystatin, 5fc, flucytosine and KTC, ketoconazole, S sensitive, I intermediate sensitive, R resistant, S/n serial number

## DISCUSSIONS

Vaginal discharge is one of most frequent gynaecological problems encountered in females, especially during their reproductive stage (Akingbade *et al.*, 2013). This study has identified the different *Candida* species responsible for various degrees of infections among female patients attending Aminu Kano Teaching Hospital. The study also determined the antifungal susceptibility profile of all the *C. species* isolated. The results for the study revealed that, the overall prevalence of *Candida* isolates in the clinical samples was (11.3%) with *C. albicans* (37.3%) as the leading specie isolated. This is in line with (12.7%) detected by (Feglo *et al.*, 2009) and (Mohanty *et al.*, 2007) who also reported as much as (18.5%) prevalence in India, among sexually active married women attending rural primary health care. And in both the studies *C. albicans* was the commonest specie isolated. The prevalence of candiduria in this study was found to be (2.3%) with *C. albicans* (37.5%) as the highest specie isolated. This is lower than the (5.1%) reported by (Ayeh-Kumi *et al.*, 2007) and (11%) observed by (Feglo *et al.*, 2009). However, isolating *C. albicans* (37.5%) as the major *C. specie* in this study has completely agreed with the findings of (Gizachew *et al.*, 2010) and (Zarei *et al.*, 2012) who equally reported (42%) and (53.3%) respectively. But (Lata *et al.*, 2012) reported that *C. glabrata* was the highest *C. spp* isolated in their studies, this has disagreed with the earlier observations that *C. albicans* was the predominant yeast specie isolated in urine samples. Although, (Robinson *et al.*, 2009) reported that candiduria in several occasions may be as result of contamination of the urine samples, urinary tract colonization or indicative of invasive urinary tract infection. Probably for these reasons, physicians have several responses to the finding of yeast in urine samples. The prevalence of *Candida species* in high vaginal swab was detected as (28.5%) and *C. albicans* (37.3%) was the most prevalent specie isolated. This is in conformity with the (28%) reported by (Garcia *et al.*, 2004) and elsewhere. Feglo *et al.*; (2009) reported (21%) prevalence in which (48.7%) of the entire isolates were *C. albicans*. Arul *et al.*, (2012) also reported (22.4%) prevalence and (64.3%) of the isolates were found to be *C. albicans*. This study has also testified the findings of (Enwaeni *et al.*, 2001; Asticcioli *et al.*, 2009; Fauzia *et al.*, 2010; Alli *et al.*, 2011 and Akingbade *et al.*, 2013) where both reported

*C. albicans* as the commonest yeast specie isolated in their studies. On the other hand, the result of antifungal susceptibility test carried out showed that resistance to nystatin was (96.1 to 100) % among the entire isolates. This is similar to the findings of (Arul *et al.*, 2012) in which all the non-*albicans Candida spp* tested in their study were completely resistant to nystatin. For ketoconazole (18.6%) of the isolates were found to be resistant. This relates with (23.6%) reported by (Fundik and Tuncer, 2002). Nevertheless, (Akortha *et al.*, 2008) observed as low as (5.8%) resistance, while (kelan *et al.*, 2011) and (Mohammed *et al.*, 2013) reported an increased resistances of (34.2 %) and (33- 50) % accordingly. The probable reason to these variations could be attributable to the availability of the drugs over the counter which encourages self – medications, coupled with long term used of the agents. This is because patients who see physician usually receive empirical therapy in which cultures are not routinely obtained and susceptibility testing is rarely performed. Fluconazole was effective against only (33.9%) of the isolates in this study. This has gone a long way with the findings of (Srinivasan and Kenneth, 2006), (Fauzia *et al.*, 2010) and (Arul *et al.*, 2012) who both reported (30%), (36.2%) and (22.2-30) % respectively. On the other hand, these results are much lower than (71.6 %) observed by (Feglo *et al.*, 2009) and (100%) by (Mohanty *et al.*, 2007). Similarly all the *C. krusei* isolated in this study were completely resistant to fluconazole, which is in line with the findings of (Feglo *et al.*, 2009) and report of (National committee on Clinical laboratory standard M44 A, 2004) which shows that *C. krusei* is inherently resistant to fluconazole. Voriconazole activity was seen in only (28.8%) of the isolates, this is slightly higher than the (18.7%) reported by (Arul *et al.*, 2012), and greatly lower than (94.7%), (97%) and (94.6%) observed by (Baran *et al.*, 2000), (Feglo *et al.*, 2009) and (Tulumoglu *et al.*, 2009) accordingly. In the same vein, susceptibility to flucytosine was detected as (25.4%). This is absolutely lower than the (96.6%) documented by (Fundik and Tuncer 2002) and (83.6%) by (Feglo *et al.*, 2009). The possible reasons to this could be suggestive to the fact that, sometimes results of in vitro antifungal susceptibility test do not always faithfully reflect events in vivo (Sobel *et al.*, 2003 and Fan *et al.*, 2007). This is due to variations among individuals, characteristics of the drugs, and variable behaviour of the microorganisms in each individual (Srinivasan and Kenneth, 2006). It is important to note that the CLSI document (M44-A) refers to disk



diffusion testing of standard conditions only for fluconazole and voriconazole. Other antifungal agents need further research to ensure the standardization of this method (Srinivasan and Kenneth, 2006).

## CONCLUSION

In conclusion, *C. albicans* has been incriminated as the leading agent responsible for candidiasis among female patients attending Aminu Kano Teaching hospital followed by *C. glabrata*. Also, the various degree of resistance observed in this research has clearly signalled the need to closely monitor candidal infections by ensuring that antifungal susceptibility test is routinely carried out for better management of patients' conditions and avoidance of emergence of resistance *Candida* strains.

## RECOMMENDATION

The need to introduce antifungal sensitivity test in Aminu Kano Teaching Hospital is of paramount importance. Development of interpreted break point for all antifungal agents using Agar diffusion method should be strongly considered by relevant bodies. Identification of *Candida* isolates to species level should be done routinely to enable brighter understanding of their distributions. Mobilization, sensitization as well as health education messages should be made public on the danger attached to self-mediations and indiscriminate use of drugs. All relevant agencies such as NAFDAC, Standard Organization and other regulatory agencies saddled with the responsibilities of checkmating the quality of drugs and food should double their effort in fighting sub-standard and fake drugs. With the present attention on yeast infections, more resources should be mobilized in order to strengthen research in the area.

## REFERENCES

- 1) Adad SJ, de Lima RV, Sawan ZT, Silva ML, de Souza MA, Saldanha JC, Falco VA, Cunha AH, Murta EF., (2001). *Sao Paulo Med J.* 119(6):200-205.
- 2) Akingbade OA, Akinjinmi AA, 4Awoderu OB, Okerentugba PO Okonko IO (2013), Prevalence of *Candida albicans* Amongst Women Attending Health Centres In Abeokuta, Ogun State, Nigeria. *New York Science Journal* 2013; 6(9)
- 3) Akortha E. E., Nwaugo V. O and Chikwe N. O., (2008). Antifungal resistance among *Candida* species from patients with genitourinary tract infection isolated in Benin City, Edo state,

- Nigeria. *African Journal of Microbiology Research Vol.* 3(11) pp. 694-699.
- 4) Alli JAO, Okonko I O, Odu NN, Kolade AF, Nwanze JC, (2011). Detection and prevalence of *Candida* isolates among patients in Ibadan, Southwestern Nigeria. *J. Microbiol. Biotech. Res.* 1 (3): 176-184
- 5) Andy IM Hoepelman, (2004). Infectious Disease – Jonathan cohen, William G-2nd edition. Harcourt publishers limited, chapter 237
- 6) Arul Sheeba Malar S., Viswanathan T., Malarvizhi A., Lavanya V. and Moorthy K., (2012). Isolation, Characterisation and Antifungal Susceptibility Pattern of *Candida albicans* and non albicans *Candida* from Integrated counseling and testing centre (ICTC) patients. *African Journal of Microbiology Research Vol.* 6(31), pp. 6039-6048
- 7) Asticcioli, S., Sacco, L., Daturi, R., Matti, C., Nucleo, E., Zara, F. and Pagani, L. (2009). "Trends in frequency and in vitro antifungal susceptibility patterns of *Candida* isolates from women attending the STD outpatients clinic of a tertiary care hospital in Northern Italy during the years 2002-2007." *New Microbiol.* 32(2): 199-204.
- 8) Ayeh-Kumi, P., Kwakye-Nuako, G., Asmah, R., Borketey, P.B., Mensah- Attipoe, I., Sowah, A.O. (2007). Parasitic infections in Ghana: Trend in urine analysis at the Korle-Bu Teaching Hospital Accra- Ghana. *Ghana Journal of Allied Health Sciences*, 1, 17-23.
- 9) Baran, J. Jr., Klauber, E., Barczak, J., Riederer, K. and Khatib, R. (2000) "Trends in Antifungal Susceptibility among *Candida* species urinary isolates from 1994 and 1998." *J. Clin. Microbiol.* 38(2): 870-1.
- 10) Bukhary, Z. A. (2008): Candiduria: A review of clinical significance and management. *Saudi journal kidney dis transpl*, 19:350-60.
- 11) Enweani IB, Gugnani HC, Okobia R, Ojo SB (2001). Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo State, Nigeria. *Rev Iberoam Micol.* 18(4):171-3
- 12) Feglo, P. K and Narkwa P., (2009): Antifungal Susceptibility of *Candida species* and *Cryptococcus neoformans* Isolated from patients at Komfo Anoyke Teaching Hospital in Kumasi Ghana.
- 13) Ferrer, J (2000). Vaginal Candidiasis : Epidemiological and Etiological Factors. *Int j gynaecol obstet*, 71. Suppl 1: S21-7.



- 14) Findik, D. and Tuncer, I. (2002). "Nosocomial fungal infections in a Teaching Hospital in Turkey: identification of the pathogens and their antifungal susceptibility patterns " *Turk. J. Med. Sci.* vol 32: 35 - 38.
- 15) Fouzia Khan, Rakhshanda Baqai (2010). In vitro antifungal sensitivity of fluconazole, clotrimazole and nystatin against vaginal candidiasis in females of childbearing age, *J Ayub Med Coll Abbottabad* 22(4).
- 16) Garcia PJ, Chavez S, Feringa B, Chiappe M, Li W, Jansen KU, Carcamo C, Holmes KK, (2004). Reproductive Tract Infections in Rural Women from the Highlands Jungle and Coastal regions of Peru. *Bull WHO* 82: 483-492.
- 17) Ghannoum, M. A. and Rice, L. B. (1999) "Antifungal agents: mode of action, mechanisms of resistance and correlation of these mechanisms with bacterial resistance." *Clin. Microbiol. Rev.* 12 (4): 501-517.
- 18) Gizachew Yismaw, Daniel Asrat, Yimtubezinash Woldeamanuel, Chandrashekhar Unaka,(2010).Prevalence of Candiduria in Diabetic Patients Attending Gondar University Hospital, Gondar, Ethiopia, *Iranian journal of kidney* (2013); 7:102-7.
- 19) Gullo A. (2009): Invasive Fungal Infections. The challenge continues, *Drugs*, 69 (suppl): 65- 73.
- 20) Kauffman, CA, Vazquez, JA, Sobel, JD, Gallis, HA, McKinsey, DS, and Karchmer, AW (2000): Prospective multicenter surveillance study of funguria in hospitalized patients. *Clin Infect Dis.* 30:14-8
- 21) Kauffman, CA. (2005). Candiduria. *Clin Infect Dis.* 41: 371- 376.
- 22) Lata R Patel, Jayshri D Pethani, Palak Bhatia, Sanjay D Rathod, Parul D Shah, (2012). Prevalence of *Candidal* infection and its antifungal susceptibility pattern in tertiary hospital, Ahmadabad. *National journal of medicine research Vol 2 issue 4*
- 23) Lundstrom T and Sobel J., (2001): Nosocomial candiduria. A review, *Clin Infect Dis.* 32:1602-1607
- 24) Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S. and Tolosa, J. E. (2007) "Prevalence and susceptibility to fluconazole of *Candida* species causing vulvovaginitis." *Indian. J. Med. Res.* 126: pp 216 -219.
- 25) Morore, M. Y., Sobel, J. D., Elhalis, H., Du, W. and Akins, R. A. (2000) "Fungicidal activity of fluconazole against *Candida albicans* in a synthetic vagina-simulative medium." *Antimicrob. Agents. Chemother.* 48(1): 161-7.
- 26) Muhammed S. Alhussaini, Noha F. El-Tahtawi, Ahmad M. Moharram, (2013). Phenotypic and Molecular Characterization of *Candida Species* in Urine Samples from Renal Failure Patients. *Science Journal of Clinical Medicine. Vol. 2, No.1, pp. 14-25.*
- 27) National Committee on Clinical Laboratory Standard (NCCLS) (2004), *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline.* NCCLS document M44-A (ISBN 1-56238-532-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- 28) Odds, F. C. (1988) *Candida* and candidosis: A review and bibliography. 2<sup>nd</sup>. Ed., London: Balliere Tindall: 68-92.
- 29) Rizvi MW, Maliki A, Shahid M, Singhal S, (2011). *Candida albicans* infections in a north Indian tertiary care hospital: Antifungal Resistance Pattern and Role of SDS PAGE for characterization, *Biology and medicine, vol 3 (2) Special issues:* 176-181.
- 30) Robinson JL, Davies HD, Barton M, O'Brien K, Simpson K, Asztalos E., (2009). Characteristics and outcome of infants with candiduria in neonatal intensive care - a Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study. *BMC Infect Dis.* 9: 183.
- 31) Saporiti, A.M. and Gomez, D. (2001). Vaginal Candidiasis: Etiology and Sensitivity Profile to Antifungal Agents in clinical use, *Rev. Argent.Microbial.* 33(4): 217-22
- 32) Schonebeck, J. and Ansehn, S. (1972). The occurrence of yeast-like fungi in the urine under normal conditions and in various types of urinary pathology. *Scand J Urol Nephrol.* 6:123-8
- 33) Shay, AC. and Miller, LG. (2004). An estimate of the incidence of Candiduria among hospitalized patients in the United States. *Infect Control Hosp Epi-demiol,* 25:894-5
- 34) Sobel, J. D., (2004) "Controversies in the diagnosis of candiduria: what is the critical colony count?" *Infect. Dis.* 4: 81-83.
- 35) Sobel, J. D., Zervos, M., Reed, B. D., Hooton, T. M., Soper, D. and Nyirjesy, P. (2003) "Fluconazole susceptibility of vaginal isolates obtained from women with complicated *Candida* vaginitis: clinical implications." *Antimicrob. Agents. Chemother.* 47: 34 – 8

- 36) Srinivasan, L. and Kenneth, J. (2006). Antibiotic susceptibility of *Candida* isolates in a tertiary care hospital in Southern India." *Indian. J. Med. Microbiol.* 24(1): page 80 -81
- 37) Walker, K., Skelton, H. and Smith, K. (2002) "Cutaneous lesions showing giant yeast forms of *Blastomyces dermatitidis*." *J. Cutan. Pathol.* 29(10): 616-8
- 38) Zarei-Mahmoudabadi A, Zarrin M, Ghanatir F, Vazirianzadeh B, (2012). Candiduria in hospitalized patients in teaching hospitals of Ahvaz, *iran jornal of microbiology vol 4 no.4* 198-202