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## Antimycotic susceptibility studies on some *Candida* species isolated among patients attending Aminu Kano Teaching Hosptial, Kano, Nigeria

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**ABSTRACT:** **Background:** Studied have established the prevalence of Vaginal Candidiasis (Moniliasis) from women attending antenatal care clinic at the Aminu Kano Teaching Hospital Kano. The antimycotic susceptibility profile has not been reported. **Objective:** To study the antimycotic susceptibility profiles of some *Candida* species isolated from suspected patients. **Materials and Methods:** Seventeen isolates of *Candida* species isolated from vaginal swabs were subjected to Germ Tube Test (GTT) for identification of *Candida albicans*. Four antifungal drugs were tested against the isolates to determine their Minimum Inhibitory Concentration (MIC) on the isolates using tube dilution method. **Results:** Miconazole had the least MIC of 0.15 mg against 41.2% of the isolates. The MIC of Ketoconazole was 0.078 against 35.3% of the isolates. Up to 29.40% of the isolates were inhibited by Nystatin and Clotrimazole at MIC of 19.53IU and 0.093mg respectively. **Conclusion:** Miconazole has more antifungal activity against *Candida albicans* isolates at the area of study.

**Key words:** Antimycotic, Susceptibility, Vaginal Candidiasis.

### Introduction

Vaginal Candidiasis simply applies to *Candida* infection of the vulvovaginal region, and this leads to the low inflammation of the area (Robert, 1991). They are the common gynecological problems, characterized by itching (Steven, 1988).. The range of infection with *Candida species* varies from a being local mucosal membrane infection to disseminated disease. There are many symptoms of vaginal Candidiasis but they vary according to the organisms or individuals (David, 2000). Symptoms of vaginal Candidiasis appear when the balance between the normal microorganisms of the vagina is lost, and the *C. albicans* population becomes large in relation to other microorganisms population (Access, 2003). This happens when the environment (vagina) has certain favourable conditions that allow growth and nourishment of *C. albicans*.

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An environment that makes it difficult for other microorganisms to survive may also cause an imbalance and lead to a yeast infection (Access, 2003). Severe disease is typically associated with an immunocompromised state including those vulnerable to iatrogenic pathogens in the intensive care unit or those with predisposing immunologic condition such as malignancy, organ dysfunction, or immunosuppressive therapy (Kauffman, 2007).

Candidiasis is a primary or secondary mycotic infection caused by members of the genus *Candida*. The clinical manifestations may be acute, subacute or chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis and meningitis. In healthy individuals *Candida* infections are usually due to impaired epithelial barrier functions and occurs in all age groups, but are most common in the new born and the elderly. They usually remain superficial and respond readily to treatment. Systemic Candidiasis is usually seen in patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppressant, or transplantation therapy (Smith, *et al*, 2005). *Candida* vaginitis remains a common problem in immunocompetent, healthy women and is predominantly caused by strains of *Candida albicans* (>90%) (Sobel, *et al*, 1998 and Sobel, *et al*, 2001). Only a minority of cases (<10%) are caused by non-*C. albicans* *Candida* species, usually *C. glabrata* and despite considerable debate, there is little evidence of a significant increase in infection rates due to the non-*C. albicans* *Candida* species (Sobel, *et al*; 1998, Sobel, *et al*, 1995 and Sobel, *et al*, 2001).

Given the reports of refractory oral and esophageal *Candidiasis* caused by azoles – resistant *C. albicans* strains and, less commonly, non-*C. Albicans* *Candida* species, it is important to monitor the azole's susceptibilities of vaginal isolates of the various *Candida* species (Fichtenbaum, *et al*; 2000, Johnson, *et al*, 1995 and Maenza, *et al*. 1997). Recently, a nationwide, multicenter prospective study was performed in which the clinical and mycological efficacies of two dosage regimens of fluconazole in women with complicated *Candida* vaginitis were compared (Sobel, *et al*, 2001). Analysis of the in-vitro azole's susceptibilities of these vaginal isolates forms the basis of this report. The susceptibilities of a large number of pathogenic isolates of *Candida* species were determined (Huvany, *et al*, 1990). Antifungal susceptibility testing remains an area of intense interest. Susceptibility testing can be used for drug discovery and epidemiology, but this study will focus on use of antifungal susceptibility testing to predict therapeutic outcome. (Arikan, *et al*. 2000). The aim of the research is to determine the antimycotic susceptibility of some *Candida* species isolated among patients attending AKTH.

## **Materials and Methods**

### **Sample Collection:**

Vaginal swabs that include Endocervical Swabs (ECS) and High Vaginal Swab (HVS) were collected by clinicians on site from the patients' by use of a sterile cotton swab for culture and microscopy.

### **Culturing**

The swab sticks were brought to the laboratory and directly inoculated on (Sabouraud's Dextrose Agar test (SDA) and Chocolate Agar (CHOC) by streaking method and the plates were incubated at 37°C for 24 hours aerobically under 10% CO<sub>2</sub> *Candida albicans* (Turk, 1983).

### **Microscopical Examination**

This was done using grease free slide where normal saline was dropped into the swab stick. When mixed, a drop of it was placed on the slide and covered with a cover slip. It was then placed under microscope objective X40 for the following:

- i. Epithelial cells.
- ii. Pus cells.
- iii. Yeast cells.
- iv. Sperm cells
- v. *Trichomonas vaginalis* (very rare).
- vi. Red blood cells.

The results obtained are recorded according to the number of them as they appeared in the preparation. Suspected cloning on both plates was Gram stained using the standard Gram staining techniques.

Many	-	+++
Few	-	++
Very few	-	+
Only one	-	+
None	-	nil

#### Germ Tube test:

Germ tube test was carried out to differentiate *Candida albicans* from other *Candida* species. After incubation *Candida albicans* was found to appeared with a germ tube like structures and this confirms their presence in that sample.

## Results

**Table 1:** Susceptibility of some antifungal drugs against some *Candida species*

Antifungal drug	MIC	Number of isolates (n=17)	% susceptible
Nystatin (IU)	19.53	5	29.41
Clotrimazole (mg)	0.093	5	29.41
Ketoconazole (mg)	0.078	6	35.29
Miconazole (mg)	0.15	7	41.17

**Table2:** Resistance of some *Candida species* against some antifungal drugs.

Antifungal drug	No. of isolates resistance at very high concentration	% resistance
Nystatin (Iu)	2/17 (625)	11.76
Clotrimazole (mg)	11/17 (0.75)	64.70
Ketoconazole (mg)	6/17 (Neat conc.)	35.29
Miconazole (mg)	7/17 (Neat conc.)	41.20

**Table 3** Germ tube test of the isolates

No of isolates	No. of GTT positive	No. of GTT Negative
17	7(41.2%)	10(58.8%)

**Table 4:** Sensitivity test of ketoconazole at different concentrations (mg)

Isolate I.D No.	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.039025	0.0195125	0.0097563
1372	-	-	-	-	-	-	+		
1239	-	-	-	-	-	-	+		
1326	-	-	-	-	-	+			
1330	-	-	-	-	-	+			
1306	-	-	-	-	-	+			
1343	-	-	-	-	-	-	-	+	
1302	-	-	-	-	-	-	-	+	
9	-	-	-	-	-	-	-	+	
1303	-	-	-	-	-	-	+		
1252	-	-	-	-	-	+			
1253	-	-	-	-	-	-	+		
1266	-	-	-	-	-	+			
1263	+	+	+	+					
1273	-	-	-	-	-	-	-	-	+
1280	-	-	-	-	-	-	-	+	
1281	+	+	+	+					
1261	-	-	-	-	+				

**Table 5:** Sensitivity test of clotrimazole at different concentrations (mg)

Isolate I.D No.	1.5	0.75	0.375	0.1875	0.09375	0.046875	0.0234375	0.0117188
1372	-	-	-	-	-	+		
1239	-	-	-	-	-	+		
1326	-	-	-	-	-	+		
1330	-	-	-	-	-	+		
1306	-	-	-	-	-	+		
1343	-	+						
1302	-	+						
9	-	+						
1303	-	+						
1252	-	+						
1253	-	+						
1266	-	-	+					
1263	-	+						
1273	-	-	-	+				
1280	-	+						
1281	-	+						
1261	-	+						

**Table 6:** Sensitivity test of miconazole at different concentrations (mg)

ISOLATE I.D. NO.	2.5	1.25	0.625	0.3125	0.15625	0.078125	0.039025	0.0195313	0.0097657	0.0048829
1372	-	-	-	-	+					
1239	-	-	+							
1326	-	-	-	-	-	-	+			
1330	-	-	-	-	-	-	-	-	-	+
1306	-	-	-	-	-	-	+			
1343	-	+								
1302	-	-	-	-	+					
9	-	-	-	+						
1303	+	+	+	+						
1252	-	-	-	-	+					
1253	-	-	+							
1266	-	-	-	-	+					
1263	-	-	-	-	-	+				
1273	-	-	-	-	-	+				
1280	-	-	-	-	-	+				
1281	-	-	-	-	-	+				
1261	-	+								

**Table 7:** Sensitivity test of Nystatin at different concentrations (I u)

ISOLATE I.D. No	5000	2500	1250	625	312.5	312.5	156.25	78.125	34.065	19.53125	4.8828125
1372	-	-	-	-	-	-	-	-	-	+	
1239	-	-	-	-	-	-	+				
1326	-	-	-	-	-	-	-	+			
1330	-	-	-	-	-	-	+				
1306	-	-	-	-	-	-	-	-	-	+	
1343	-	-	-	+							
1302	-	-	-	+							
9	-	-	-	-	-	-	-	-	-	+	
1303	-	-	-	-	-	-	-	-	-	+	
1252	-	-	-	-	-	-	-	-	+		
1253	-	-	-	-	+						
1266	-	-	-	-	-	-	-	-	-	-	+
1263	-	-	-	-	-	-	-	-	+		
1273	-	-	-	-	-	-	-	-	+		
1280	-	-	-	-	-	-	-	+			
1281	-	-	-	-	-	-	-	-	+		
1261	-	-	-	-	-	+					

## Discussion

Vaginal Candidiasis is one of the major infections of vaginal region; it is caused by *Candida albicans* species (Milton, 1979). The organism probably gets to the vagina from the fungal infection that affects or result from the intestinal tract, or it can be spread by several means (David, 2000). Some fungi, especially yeasts and yeast like ones have been associated with vaginal Candidiasis, and the most important and predominant specie in this research was found to be *Candida albicans*. This is in line with the work of Horowitz, (1989) in which 33 vaginal cultures revealed that the most predominant was *C. albicans*. Also the prevalence of Candidiasis in women between the ages of 15 – 20 was found to have the highest prevalence of the infections. This is due to their reproductive years as stated by Boyd, (1991). This also is in corroboration with the work of Miline and Warrock (1979).

In addition, vaginal *Candida* colonization is also associated with different types of clinical manifestation which may result in Candidiasis or the infection may occur as a result of their association. They include among others pelvic inflammatory disease (PID), which is the most predominant in the work. Vaginal discharge, itching, sexually transmitted diseases (STD), infertility cases, Dysuria e.t.c. are some of the other manifestations as reported by David, (2000). This also agrees with the work of Valdimerson *et al*, (1973) in which all these manifestations were reported.

The result of the study demonstrated that, the sensitivity pattern of varying concentrations of the antifungal (Nystatin, clotrimazole, ketoconazole and miconazole) on some clinical *Candida* isolates have shown an occasional variation in their minimum inhibitory concentration (MIC) from the result, about 29.41% of the isolates were susceptible to nystatin at very low concentration and 11.76% were resistant at very high concentrate; which shows that, nystatin is very effective to most of the *Candida* isolates. Also as demonstrated, 29.41% of the isolates were susceptible to clotrimazole at lower concentration with 64.70% being resistant at very high concentration this shows that clotrimazole is not effective to most of the isolates. However, some of the isolates show resistance even at neat concentration of ketoconazole and miconazole while 35.29% and 41.70% of the isolates become susceptible at very lower concentration of the drugs respectively. From table 3, it can be interpreted that, most of the isolates are non-*albicans* being having the highest percentage of the germ tube test to be negative.

## Conclusion

The antimycotic susceptibility of *Candida* species isolated among patients attending AKTH was determined. It can be concluded that, Candidiasis is the major infection of the vulvovaginal region, and *Candida albicans* is the major *Candida* specie that causes it. Different type of clinical manifestations or provisional diagnosis were found to be associated with the infection, Therefore, care has to be taken when symptoms of such infections manifest so that routine diagnosis could be made to identify the sole causes of the infection before treatments.

## Recommendations

From the study, it can be recommended that, prescription of clotrimazole should be stopped because it is not a drug of choice. However nystatin, ketoconazole and miconazole can still be maintained in treating *Candida* vaginitis because they are efficacious.

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