FLUCONAZOLE RESISTANCE IN CLINICAL ISOLATES OF *Candida albicans* IN SOME SELECTED HOSPITALS IN SOKOTO METROPOLIS

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ABSTRACT

Aim: The aim of the study is to determine fluconazole resistance in clinical isolates of *Candida albicans*in some selected hospitals in Sokoto metropolis

Methods: A total of 170 samples were collected consisting of oral swabs, high vaginal swab (HVS) and endocervical swab (ECS). Standard mycological analyses such as culture on chromogenic agar, Germ tube test and antifungal susceptibility testing were carried out to isolate and identify *C. albicans*

Results: The most prevalent yeast isolated was *C. albicans* (41.2%) followed by *C. krusei*(17.6%), *C.tropicalis* (12.9%) and *C. glabrata* (1.2%). Prevalence of resistance and susceptibility were 34.3% and 65.7% respectively. Prevalence of resistance was higher in isolates from females (38.5%), age group 41-50 (100%) and ECS (50%).

Conclusion: In this study, fluconazole resistant *C.albicans* is prevalent in Sokoto metropolis and there is need to review antibiotic policy.

Keywords: *C. albicans*, fluconazole, Sokoto, resistance, ECS,HVS

INTRODUCTION

Candida is a genus of yeasts and is the most common cause of fungal infections worldwide (Manokolakaki*et al.,*2010). They are small, oval and measuring 2-4 micrometer in diameter, the yeast is Gram positive when stained with Grams stain. Many species are harmless commmensalsof humans; however when mucosal barriers are disrupted or the immune system is compromised they can invade and cause a disease called Candidiasis (Kourkoumpetis and Thermistoklis, 2011). *Candida albicans* which is the major species is a normal flora found in the mouth, vagina and gastrointestinal tract in 40-60% healthy adults (Kerawala and Newland, 2010).

Itis the most commonly isolated species and can cause infections (*candidiasis*or thrush) in humans and other animals (Fugelsang and Edward, 2010). *Candida albicans* is an important nosocomial pathogen and can be transmitted sexually (Tatfeng*et al.,* 2004). When grown in the lab it appears as large, round, white, or cream colonies, which emits yeasty odour, *C.albicans* ferments glucose and maltose to acid and gas, it does not ferment lactose which helps to distinguish it from other *Candida* species (Edward *et al.,*1978).

There are 3 major forms of candidiasis: oropharyngeal candidiasis*,* vulvovaginal candidiasis and invasive candidiasis. For oropharyngeal candidiasis, infection occurs in the mouth or throat and is identified by white plaque growth on oral mucous membranes, vulvovaginal candidiasis is the overgrowth of *C.albicans* in the vagina, and results in rashes, itching and discharge from the genital region and invasive candidiasis occurs when Candida enters the blood stream and can easily spread to organs throughout the body. *C albicans* has virulent factors like Adhesins, invasions, biofilm formation and hydrolases which aids in its pathogenicity.

Antifungal agents have greatly contributed to the improvement of public health; *C.albicans* infections are usually treatable with fluconazole.

Fluconazole act by inhibiting egosterol synthesis, which is the predominant sterol in fungal plasma membranes; it is important for membrane integrity and for the activity of many membrane-bound enzymes (Hitcock, 1991).

Nevertheless, antifungal resistant pathogens have increased during the past decades, becoming serious concern.The increasing antifungal resistance is due to the inappropriate usage and due to the use of over the counter antifungal agents widely used in developing countries (Kavitha*et al.,* 2017). Since fluconazole is used commonly for treating Candidiasis, fluconazole resistant *Candida albicans* is an emerging problem nowadays leading to morbidity and mortality (Kavitha*et al.,* 2017).

Over 75% of women suffer from a *C.albicans* infection, usually vulvovaginal Candidiasis in their lifetimes, and 40%-50% of them have additional occurrence(s). *C.albicans* is the 4th leading cause of nosocomial infections, this result in an extremely life-threatening systemic infection in hospitalized patients with mortality rate of 30% (Pfaller and Diekema, 2007). In the United States, Orophryngeal colonization by *C.albicans* can be found in almost 30%-55% of young adults (Hidalgo *et al.,* 2014). In HIV patients, over 90% develop a case of Oropharyngeal Candidiasis (*de* Repentatigny*et al.,* 2004).

Approximately 7% of *Candida albicans* are resistant to fluconazole (Lockhart *et al.,* 2012 and Vallabhaneni*et al.,* 2014). In 2013 the United States Centres for Disease Control (CDC) reported that fluconazole resistant *Candida albicans* poses a serious threat and is responsible for approximately 3,400 cases annually (CDC, 2013).Data obtained from this study may help in the development of new therapeutic approaches; new antifungals or modify existing antifungals with reduced resistance so as to provide effective treatment on fluconazole resistant *C.albicans* and reduce cost of ineffective treatment with fluconazole.

MATERIALS AND METHODS

Study Area

This study was carried out in Sokoto state which is located within the North-Western geopolitical zone of Nigeria. According to the National Population Commission (2010), population figures stand at 3,7026,76 persons with a land area of 33,776.89 square kilometres. The population mainly consists of the Hausa/Fulani ethnic groups; the major occupation of the people is farming and animal husbandry. The two major seasons in the State are the dry (October to May) and wet seasons (May to October). Majority of its indigenes are Muslims (UNFPA, 2013).

The study was carried out in two hospitals within Sokoto Metropolis; Specialist Hospital Sokoto and Maryam Abacha Women and Children Hospital Sokoto. These hospitals span within two Local Government Areas; Sokoto North and Sokoto South Local Government Areas of Sokoto State respectively.

Study population

Oral swabs, High vaginal swabs and Endocervical swabs of patients.

Sample size determination

Using the formulae; $n = \frac{Z^2Pq}{d^2}$ (Charan and Biswas, 2013)

Where:

n= Minimum number of samples required (sample size)

Z= Standard deviation at 95% confidence interval = 1.96

P = Prevalence from initial studies = 4.3% = 0.043 (Akortha*et al.,* 2009)

d = degree of confidence = 5%= 0.05

q = 1-p = 1-0.043 = 0.957

susbstituting the data in the formulae

$$n = \frac{1.96^2 \times 0.043 \times 0.957}{0.05^2} = \frac{3.8416 \times 0.043 \times 0.957}{0.0025} n = 63$$

The samples size was increased during the work to 170 in order to increase chances of *C. albicans* isolation.

Sample collection

Oral swabs were collected by rolling a sterile swab stick on the surface of the subject's tongue and side of the cheeks. High vaginal swabs (HVS) and endocervical swabs (ECS)were collected with the aid of a sterile speculum.

Informed consent

Informed consent was sought from the target subjects, and the type of test to be carried out was explained to them in their languages. Only subjects willing to give sample were used for the study.

Ethical consideration

Ethical permission was obtained from the ethical committees of the two selected hospitals.

Mycological analysis

Wet Preparation

A drop of normal saline was placed into the enclosed swab stick and allowed to stand for 1 minute to dissolve the sample. A drop from the dissolved sample was placed on clean grease free glass slide and covered with a cover slip and viewed under microscope using ×10 and ×40 magnifications.

Culture

Samples were cultured on Chromogenic Candida agar (Oxoid) and incubated at 37° C. Inoculated plates were examined after 48 hours of incubation.*Candida albicans* grow asgreen colonies, *C. tropicalis* (dark blue colonies), *C. krusei* (brown colonies) and *C. glabrata* (yellow colonies) (Sumitra and Megha, 2014). Isolates were subcultured on Sabouraud dextrose agar (SDA) and incubated at 37^0C for 48 hours; cream coloured pasty colonies with distinctive yeast smell were observed (Cheesebrough, 2006).

Gram staining

A drop of normal saline was placed on a clean grease free glass slide; a smear was made by emulsifying a colony on the drop of normal saline using a sterile wire loop. It was allowed to air dry and heat fixed using flame, the dried smear was placed on a staining rack and flooded with crystal violet and allowed to stain for 1 minutes, it was washed with water and then covered with lugol's iodine to stain for 1 minute, then washed with water and decolourised with 1% acetone briefly. The smear was then stained with neutral red for 1 minute washed with water, drained and allowed to dry then viewed under oil immersion objective. Yeasts appears Gram positive (Sumitra and Megha, 2014).

Germ tube test (GTT)

A sterile Pasteur pipette was used to pick 0.5ml of sterile bovine serum and placed into a test tube; a light suspension of suspected yeast colonies was prepared. It was incubated for 2-3 hours at 37^0C in a water bath. A Pasteur pipette was used to transfer a drop of the serum yeast culture to clean grease free glass slide and covered with a cover slip ansd examined microscopically for the production of germ tubes. Germ tubes appear as sprouting yeast cells, that is tube-like out-growths from the cells (Cheesebrough, 2006).

Antifungal Susceptibility testing

Antifungal susceptibility testing of the isolates was carried out using disk diffusion method on Mueller-Hinton Agar supplemented with 2% Glucose and 0.5ug/ml Methylene Blue Dye (Ghandi *et al.,* 2015). About five distinct colonies were picked from the *Candida albicans* isolates and suspended in 5.0 ml of sterile saline (0.85 g/L NaCl) and mixed thoroughly on a vortex mixer, the suspension was adjusted to 0.5 McFarland turbidity Standard (10^6 CFU/ml) using Spectrophotometer (NCCLS, 2008). A sterile cotton swab was dipped into the suspension, the swab was pressed firmly against the inside wall of the tube above the fluid level to remove excess fluid from the swab.The dried surface of a sterile Mueller-Hinton agar plate was inoculated by evenly streaking the swab over the entire agar surface.

A disk containing 25ug fluconazole was placed on each inoculated plate using flamed forceps, the plates were incubated at 37^0C for 24 hours and the diameter of the zones of inhibition was measured.

Inhibitory zone of fluconazole was measured at transitional point where growth abruptly decreases, determined by mark reduction in colony sizes (William *et al.,* 1998). The standard diameter for fluconazole susceptibility and resistance used are: diameter of ≥ 19mm and diameter of ≤ 14mm respectively (NCCLS, 2008).

3.10 Statistical analysis

The data collected was presented using tables, percentages and Statistical Package for Social Sciences (SPSS) Windows version 21.0, the degree of confidence was set at 95% (P-value of 0.05).

RESULTS

Table 1 shows the distribution and isolation of Candida species according to study sites, it also shows the total number of samples collected and the prevalence of *Candida albicans* and other *Candida* species among samples. 144 samples were collected from Specialist hospital and 26 samples were collected from Maryam Abacha hospital Sokoto. The most prevalent yeast isolated was *Candida albicans*(41.2%) followed by *C.krusei*(17.6%), *C. tropicalis* (12.9%), and *C. glabrata* (1.2%).

Table 1:Distribution and isolation rate of Candida species from the studied hospitals.

Hospitals	Samples Examined	*Candida albicans* (%)	*C. krusei*(%)	*C. tropicalis* (%)	*C. glabrata* (%)
SHS	144	63 (43.8)	26 (18.1)	19 (13.2)	0 (0)
MWCH	26	7 (26.9)	4(15.4)	3 (11.5)	2(7.7)
Total	**170**	**70 (41.2)**	**30 (17.6)**	**22 (12.9)**	**2 (1.2)**

Key: SHS = Specialist Hospital Sokoto
MWCH = Maryam Abacha Women and Children Hospital

Table 2 describes the antibiogram pattern of *C.albicans* isolates based on type of sample; it also shows the distribution of *C.albicans* based on nature of specimen. Of the 70 *C.albicans* isolates obtained 46 were isolated from oral swabs, 22 from HVS and 2 from ECS. ECS has the highest prevalence of fluconazole resistant *C.albicans* (50%) followed by oral swabs (34.8%) and HVS (31.8%). For susceptible isolates HVS has the highest prevalence of 68.2%, followed by oral swabs 65.2% then ECS 50.0%. There is no significant association between sample type and susceptibility or resistance to fluconazole (P > 0.05).

Table 2: Shows the susceptibility and resistance of *C.albicans* isolates to Fluconazole based on sample type.

Type of sample	Susceptible (%)	Resistant (%)	Total	P-value
				0.868
Oral swab	30 (65.2)	16 (34.8)	46	
High Vaginal Swab	15 (68.2)	7 (31.8)	22	
Endocervical Swab	1 (50.0)	1 (50.0)	2	
Total	**46 (65.7)**	**24 (34.3)**	**70**	

χ^2= 0.284

Depicted in table 4.3 is the distribution of fluconazole susceptible *C.albicans* and fluconazole resistant *C.albicans* based on Gender. 39 isolates from the total 70 were from females and 31 were from males, of the 39 isolates from females 15 (38.5%) were resistant and 24 were susceptible (61.5%). 9 (29.0%) of the 31 isolates from males were resistant to fluconazole while 22 were susceptible (71.0%). There is no significant association between gender and distribution of resistance or susceptibility of the isolates (P>0.05).

Table 3: Shows the susceptibility profile of *C.albicans* to Fluconazole based on Gender.

Gender	Susceptible (%)	Resistant (%)	Total	P-value
				0.409
Female	24 (61.5)	15 (38.5)	39	
Male	22 (71.0)	9 (29.0)	31	
Total	**46 (65.7)**	**24 (34.3)**	**70**	

χ^2= 0.682

Distribution pattern of susceptible and resistant isolates based on age of participants is demonstrated in Table 4. The age range of participants was between 0-50 years. The age group with the highest resistance rate was 41-50 years with a prevalence of 100%, this was followed by the age group 11-20 years (50.0%), 31-40 years (40.0%), 0-10 years (34.8%) and 21-30 years (21.4%). This variation was found not to be statistically significant with a (P>0.05).

Table 4: Shows the susceptibility profile of *C.albicans* isolates to Fluconazole based on the age of participants.

Age (years)	Susceptible (%)	Resistant (%)	Total	P-value
				0.484
0-10	30 (65.0)	16 (34.8)	46	
11-20	2 (50.0)	2 (50.0)	4	
21-30	11 (78.6)	3 (21.4)	14	
31-40	3 (60.0)	2 (40.0)	5	
41-50	0 (0.0)	1 (100.0)	1	
Total	**46 (65.7)**	**24 (34.3)**	**70**	

χ^2= 3.460

Table 5 shows the pattern of distribution of resistant and susceptible isolates to fluconazole based on hospitals. Of the 63 isolates obtained from Specialist hospital Sokoto 21 were resistant to fluconazole (25µg) with a prevalence of 33.3% while 42 were susceptible with a prevalence of 66.7%, among the 7 isolates obtained from Maryam Abacha women and Children Hospital Sokoto 3 (42.9%) were resistant and 4 (57.1%) were susceptible. The prevalence of susceptibility and resistance in the two hospitals is 65.7% and 34.3% respectively. The variation was found not to be statistically significant with a (P>0.05).

Table 5: The susceptibility and resistance of *C.albicans* isolates to Fluconazole based on hospital.

Hospital	Susceptible (%)	Resistant (%)	Total	P-value
				0.615
Specialists Hospital, Sokoto	42 (66.7)	21 (33.3)	63	
Maryam Abacha Women and Children Hospital	4 (57.1)	3 (42.9)	7	
Total	**46 (65.7)**	**24 (34.3)**	**70**	

χ^2= 0.254

DISCUSSION

This study was designed to isolate and determine the distribution of fluconazole resistant *Candida albicans*. A total of 170 samples were collected and a prevalence of 41.2%of *C. albicans*was established. This is in agreement with findings of Enwuru*et al.* (2008) andTaura*et al.*(2013) who reported prevalence of 40.5% in Lagos and 48.4% in Kano respectively. However lower prevalence 26.0% in Nassarawa, 28% and 27.9% in other parts of Africa were reported by Maikenti*et al.,* 2016; Muvunyi and Hernandez, 2009; Felgo and Narkwa, 2012, respectively. Higher prevalence has also been reported in some studies; 77.0% by Oyewole*et al.*(2010) among HIV- infected patients in Sagamu, and 70.0% reported by Nwakwo*et al.*(2010) among females of reproductive age in Kano. This is an indication that prevalence of *Candida albicans*probably varies from region to region. Increased prevalence of Candidiasis can also be as a result of frequent visit to hospitals, improper personal hygiene, inappropriate use of antibiotics, immunosuppression, and uncontrolled diabetes mellitus (Nsofor*et al.,* 2016).

The prevalence of fluconazole resistant *Candida albicans* in this study was 24(34.3%) out of 70 *C. albicans* isolates, and the prevalence of susceptibility was 46 (65.7%). Similar findings with prevalence rate of 36.4% and 32% fluconazole resistant *C. albicans*werereported by (Kaur *et al.,* 2016 and Kavitha*et al.,* 2017) respectively. However lower prevalence has been reported by previous studies. This includes 4.3% by Akortha*et al.* (2009) in Edo and 3.6% by Taura*et al.*(2013). The prevalence rate (34.3%) in this study indicates that fluconazole resistance is on the increase compared to previous studies with lower prevalence.

C. albicans isolates from endocervical swabs (50%) have the highest resistance, followed by isolates from oral swabs (34.8%) and the least areisolates high vaginal swabs (31.8%). This finding disagrees with findings of Enwuru*et al.* (2008) who reported 10% of oropharyngeal candidiasis patients and Masri*et al.* (2015)who reported 0% in high vaginal swabs in Malaysia. A lower prevalence (3.6%) in endocervical swabs and high vaginal swabs were also reported by Taura*et al.* (2013).

Prevalence of resistance of *C. albicans* isolates was higher (38.5%) in females than males (29%) which are in agreement with the findings of Kavitha*et al.*(2017) who reported 32% in females and 30% in males. The difference in figure between the two studies may be due to the number of samples collected. Prevalence of resistance of isolates in females may be due to the fact that women visit hospitals more than men due to obvious reasons of antenatal care and complications that may arise due to childbearing.

Others include hormonal changes in females, use of tight and nylon underwear, improper personal hygiene such as cleansing their vagina from back to front instead of from front to back after using the toilet, as well as use of vaginal douches (Nsofor*et al.,* 2016).

Candida albicans isolates from age group of 41-50 years shows highest resistance (100%) followed by the age group 11-20 (50%), 31-40 (40%), 0-10 (34.8%) and 21-30 shows the lowest resistance (21.4%).The reason behind prevalence of resistance being high among age group 41-50 years may be due to small sample size used in this study the likely age group to have highest prevalence is age group 31-40 (40%) which agrees with the findings of Kavitha*et al.*(2017) who reported 25-45 (73%) as the highest in a study carried out in India. The variation in prevalence between the two studies may be due to the small sample size in this study. Prevalence in this age group may be due to the lowlevels of protective cervical antibodies, increased sexual activity and influence of reproductive hormones.

The risk factors in this age group include use of oral contraceptive pills, intra uterine devices, broad spectrum antibiotics and diabetes mellitus.

Prevalence among 0-10 (34.8%) may be due poor oral hygiene and low immune status. The variation is not statistically significant (P>0.05) indicating that age is not a factor in colonization with fluconazole resistant *C. albicans*.

Maryam Abacha has the highest prevalence of fluconazole resistant *C. Albicans* with 42.9% than Specialist hospital with 33.3% in this study. This may probably be because patients that attend Specialist hospital have higher socioeconomic status. *C. albicans*is a nosocomial organism and Specialist hospital has a cleaner environment and better infection control measures.

CONCLUSION

In this study *C. albicans* was isolated and identified with a prevalence of 41.2%.The susceptibility test indicated that 34.3% of the *C. albicans* isolates were resistant to fluconazole. Maryam Abacha hospital has the highest prevalence of fluconazole resistant isolates, females harbour more fluconazole resistant *C. albicans*, age group 41-50 (100%) has the highest and ECS was found to have more fluconazole resistant *C.albicans* isolates. In conclusion this study demonstrated that fluconazole resistant *C. albicans*is prevalent in Sokoto.

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