ISOLATION and ANTIFUNGAL SUSCEPTIBILITY PATTERN of Candida albicans ISOLATED from ENDOCERVICAL and HIGH VAGINAL SWABS of PREGNANT WOMEN ATTENDING STATE SPECIALIST HOSPITAL GOMBE, NIGERIA.

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ABSTRACT
Candida albicans is the common cause of both oral and vaginal candidiasis in humans. This candidiasis leads to a wide range of physical, psychological and even physiological problems in humans particularly pregnant women. Samples of endocervical and high vaginal swab were collected from 200 women attending Gombe specialist hospital Gombe and inoculated on Saboraud Dextrose Agar (SDA) incorporated with Chloramphenicol to get rid of the unwanted bacterial contaminants. Gram staining technique and Germ tube test were employed for the identification, as, Candida albicans is positive for both. The gram positive samples were 70% (n=140) and were further subjected to germ tube test. The remaining 30% (n=60) were found to be gram negative. 90% (n=126) of the gram positive ones isolated, were also found to be positive for germ tube test; confirming the presence of Candida albicans. Antifungal susceptibility testing revealed that members of Imidazoles (Ketoconazole, Miconazole) and those of Triazoles (Fluconazole and Itraconazole) were found to be more effective at concentrations of 20, 50 and 100µg/disc than Griseofulvin (Fulcin) with only 26.00mm zone of inhibition at 100µg/disc concentration.

Keywords: Candida albicans, endocervical, antifungal susceptibility, Imidazoles, Triazoles.

INTRODUCTION
The species of the genus Candida are the cause of vaginal mycosis called vaginal candidiasis. The vulvovaginal candidiasis is one of the most common vaginal infections in women, in the fertile period and also the most frequent and most important fungal disease of vaginal content (Sobel, 1996). Women around the world get diagnosed of vaginal candidiasis. It is estimated that 75% of women during the fertile period have at least one episode of vaginal candidiasis in their lifetime. Approximately 40-50% women have repeated infections. Less than 5% of adult female population receives repeated attack of recurrent vulvovaginal candidiasis. Point-prevalence indicates that Candida species may be isolated from the genital tract of approximately 20% (range 10-15%) of asymptomatic health women who are culture positive for Candida species in the vaginal area are asymptomatic carriers (Mirela et al., 2010). The natural history of asymptomatic colonization is unknown, although limited human studies suggest that vaginal carriage may continue for several months and perhaps, years (Mirela et al., 2010). Several species of the yeast genus Candida are capable of causing candidiasis. They are members of the normal flora of the skin, mucous membrane and gastrointestinal tract. Candida species colonize the mucosal surfaces of all humans during, or soon after birth, and the risk of endogenous infection is ever present. Candidiasis is the most common systemic mycosis (Geo et al., 2001). Vulvovaginal candidiasis caused by the fungus, C. albicans is approximately 85% of cases, while other species such as C. glabrata, C. krusei, C. tropicalis and C. stellioidea rarely cause vaginitis (Van Dycket al., 1999).
Although vaginal candidiasis is both treatable and mild, if left untreated, is a possible risk for acquisition of HIV/AIDS as well as other complications (UNAIDS, 2003). Other complications include pelvic inflammatory disease, menstrual disorders, spontaneous abortion and premature birth. It is now well established that the presence of infective vaginal discharge greatly facilitates transmission and acquisition of HIV between sexual partners (FMOH, 2005, Abebe et al., 2001).

Candida species in the vaginal mucosa was found in 35% of healthy women (Goldacre et al., 1979). Numerous studies worldwide show that C. albicans are responsible for the greatest number of symptoms associated with the vaginal candidiasis (Mirela et al., 2010). In the past three decades, report shows that there had been an increased percentage of infections caused by non-albican species. These non-albican species are often resistant to conventional therapy (Babic et al., 2004).

Numerous studies indicated that pregnancy is indeed a risk factor and favours the formation of infection and its higher frequency (Sobel et al., 1998). It was demonstrated that oestrogen increases the affinity for the vaginal epithelial cell adherence of candida and yeast cytosol receptor or system to connect to reproductive hormones; these hormones also increase the formation of yeast blastophores as their morphological form.

The incidence of candida vulvovaginitis caused by non-albican species increased during the last decade (Kent et al., 1991). According to Sobelet al., 1998, studies indicated that a number of risk factors enhance the ability of non-albican species to cause infections. These include the uncontrolled use of antifungal agent, incomplete and prolonged use of antifungal agent in the prevention of candida infection. Odds et al. (1998) indicated that the occurrence of vulvovaginitis candidiasis attributed to non albicans species increased from 9.9% in the 1970s to 20.3% in the 1980s. C. glabrata, C. tropicalis and C. krusei are listed as the most frequently detected species. It is believed that occurrence of these species is associated chronic vaginal candidiasis.

Systemic conditions such as diabetes mellitus, HIV/AIDS, organ transplant and any chronic debilitating illness can increase the women chances of developing vulvovaginal candidiasis (John, 2000). Depressed cell mediated immunity provides a favourable condition for growth of candida species such as in HIV/AIDS, whereas dysfunction of neutrophils and monocytes favours candidal growth in diabetes mellitus. Broad spectrum antibiotics users posed a 16% risk of vulvovaginal candidiasis, antibiotics and vaginal douching that suppress normal bacterial flora can allow candida organism to proliferate (Nwadioha et al., 2010). Of interest is that, sulphonamides decrease neutrophils, intracellular killing of candida organism, and tetracycline and Aminoglycosides have been shown to decrease neutrophil phagocytosis (John, 2000), while oestrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of C. albicans, and also decreases immunoglobulin in vaginal secretions resulting in increased vulnerability of pregnant women to vaginal candidiasis (Fidel et al., 2000).

In addition, other contributing factors to predispose women to acute and chronic vulvovaginal candidiasis include HIV status (Dueret et al., 1990), the use of contraceptives (Sobelet al., 1998), reproductive hormones (Fidel et al., 2000 and Nohmi, 1995), contraceptive diabetes and frequent antibiotic therapy (Xu and Sobel, 2004).

The aim of this research therefore was to study the incidence of C. albicans from the endocervical and vaginal secretions (swabs) among pregnant women attending Gombe specialist hospital, Gombe with a view to screening the pregnant women for the incidence of vaginal candidiasis and finding out a possibly better antifungal agent among imidazoles and other antifungal agents to cure the ailment caused by C. albicans by in-vitro antifungal susceptibility testing.

**MATERIALS AND METHODS**

**Sampling site**

The sampling site was the Gombe state specialist hospital situated in Gombe metropolis, Gombe state, a state in the south eastern Nigeria. Women of age range of 18-45 years were clinically assessed. They were chosen because they are within the range of high risk group, are sexually active and as well, are the most vulnerable because of the nature of their immune systems (John, 2000).
Sample Collection
Endocervical and high vaginal swabs were aseptically collected with the aid of sterile swab sticks (Van Dyck et al., 1999) from pregnant women attending Specialist Hospital Gombe. The swab sticks containing the samples were transported to the Microbiology laboratory, Gombe state university, Gombe, for analysis.

Microscopy
The swab samples obtained from the vaginal discharge were covered with a coverslip on a glass slide and microscopically examined using x400 (Nwadioha et al., 2010) magnifications for the examination of macro and microconidia of yeast cells therein.

Culture
A culture of the yeast isolates (Candida species) was obtained following the streaking method of inoculation as described by Cheesbrough (2000) and Tortora et al. (2007). The swab sticks containing the endocervical and high vaginal swabs obtained from the hospital were streaked onto the plates of Saboraud Dextrose Agar (SDA) treated with antibiotic, Chloramphenicol to rid the medium of bacterial contaminants. The plates were incubated at 37°C for 24 hours and then for two days. Colonies of yeast cells that looked opaque white to creamy colour, typical of *C. albicans*, were observed as demonstrated by Cheesbrough (2000).

Plates incubated were read as demonstrated by Cheesbrough (2000) based on the colonial characteristics as: size, shape, colour, edge, consistency and diameter.

Culture Plate Reading
Plates were read after 24 hours of inoculation as demonstrated by Cheesbrough (2000). The reading was based on the colonial characteristics such as:
- Size. Tiny or large colonies
- Shape. Circular, round irregular or fimbriated
- Colour. Creamy, red, greenish or black colonies
- Edge. Entire or crenated
- Consistency. Mucoid or non-mucoid
- Diameter. 0.5mm.

Gram’s staining
Small amounts of the fungal colonies were smeared on glass slides and gram stained for observation under the microscope as described by Cheesbrough (2000).

Confirmatory Germ Tube Test
The procedure described by Cheesbrough (2000), was employed. 500μl (0.5ml) of human serum was pipetted into a small test tube. Using a sterile wire loop the yeast colony was inoculated in the serum and then placed in an incubator at 35°C-37°C for 2-3 hours. Using a Pasteur pipette, a drop of serum yeast was placed on a glass slide, and covered with a cover slip. The preparation was examined using x10 and x40 objectives with the condenser iris diaphragm closed sufficiently to give good contrast. Sprouting yeast cells that are with tube-like outgrowths observed indicated positive germ tube test.

Standardization of Inoculum
Two to three colonies of confirmed *Candida albicans* isolates were picked and incubated into 10ml of a broth incorporated with Chloramphenicol in a test tube. The test tube was then incubated at 37°C overnight at ambient air as demonstrated by Mukhtar and Okafor (2002). The overnight broth culture was further diluted with sterile normal saline to a turbidity that matched 0.5McFarland standard Mackie and McCartney, (1991), Cheesbrough, 2000).

Preparation of Sensitivity Discs and Bioassay Procedure
Sensitivity discs of 6mm in diameter were punched out of Whatman’s No. 1 filter paper and 100 discs were put in Bijou bottles, sterilized and kept for further use. 1mg(1,000μg)/ml, 2mg(2,000μg)/ml, 5mg(5,000μg)/ml and 10mg(10,000μg)/ml of the antifungal agents (Ketoconazole, Miconazole, Fluconazole, Itraconazole and Griseofulvin were prepared using) sterile deionized distilled water as the diluent. These were dispensed in the Bijou bottles containing the filter paper discs and kept at 4°C in the refrigerator for further analysis. The isolates positive for *C. albicans* were subcultured onto the prepared SDA and the plates streaked to a thin smear. The antifungal antibiotic discs were aseptically placed onto the inoculated culture media using sterile forceps and incubated at 37°C for 24 hours. The zones of inhibition were finally recorded.
Results and Discussion

**TABLE 1: Gram reaction of the fungal isolates:**

<table>
<thead>
<tr>
<th>Gram Reaction</th>
<th>Number of samples observed</th>
<th>Percentage occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>140</td>
<td>70%</td>
</tr>
<tr>
<td>Negative</td>
<td>60</td>
<td>30%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100%</td>
</tr>
</tbody>
</table>

The Table above summarizes the Gram staining reaction of 200 samples obtained from 200 pregnant women which indicated that 140 (70%) of the samples tested positive and 60 (30%) proved negative. The presence of Gram positive ones paved a way for the presence of *C. albicans* which is Gram positive and this therefore, led to the confirmatory germ tube test.

**Table 2: Incidence of *Candida albicans* using confirmatory germ tube test:**

<table>
<thead>
<tr>
<th>Germ tube test</th>
<th>Number of samples observed</th>
<th>Percentage occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>126</td>
<td>90%</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>10%</td>
</tr>
<tr>
<td>Total</td>
<td>140</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2 shows the result of the germ tube test of the 140 gram positive fungal isolates out of which 126 were positive for germ tube test, equivalent to 90% and 14 samples were negative for germ tube which is also equivalent to 10%.

**Table 3: Sensitivity of *C. albicans* to some antifungal agents tested:**

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>Concentration (µg/disc)</th>
<th>Zones of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.00</td>
<td>18.50</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>10.50</td>
<td>21.00</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.00</td>
<td>18.00</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3 displays the sensitivity pattern of the organism to the agents in which both were inactive at 10µg/disc concentration except Fluconazole.

In Table 1 the Gram's staining reaction of 200 fungal isolates obtained from specialist hospital indicated that 140 of them were Gram positive (70%) and 60 were Gram negative (30%). The result gave a clue of the presence of *C. albicans* which is Gram positive, and, this further called for the germ tube test for confirmation.

Table 2 shows the result of the germ tube test of the 140 gram positive fungal isolates out of which 126 were positive for germ tube test, which account for 90% of the 140 gram positive isolates. 14 samples were germ tube negative, equivalent to 10% (Fig. 1). This corresponds with the work of Nwadioha et al. (2010) who reported that out of 420 candida isolated, 354 (84%) were *C. albicans*. It is also in line with the study of Van Dyck et al., 1999 who reported that vulvovaginal candidiasis is created by the fungus *C. albicans* approximately by 85%, with *C. glabrata* responsible for the remaining 15%.

In table 3, at 10µg/disc concentration, all the antifungal agents with the exception of Fluconazole showed no activity. At the concentrations of 20, 50 and 100µg/disc however, the zones of inhibition were found recorded for all other antifungal agents except Griseofulvin, which indicated an activity only at the concentration of 100µg/disc. Other concentrations of Griseofulvin indicated no activity as seen in Fig. 2. This is owing to the fact that Ketoconazole, Fluconazole and Itraconazole are useful in the treatment of both vaginal and mucocutaneous candidiasis due to their good solubility and are systemically easily absorbable.

However, as said earlier, Griseofulvin showed no activity at all the concentrations except at 100µg/disc in which 26.00mm was recorded as the zone of inhibition (Table 3).
This must not be unconnected with the fact that Griseofulvin is poorly absorbed and concentrated in the stratum corneum when administered and is only clinically useful in the treatment of dermatophytes infections of the skin, hair and nails and not for the genitourinary tract fungal infections (Geo et al., 2001).

Fig. 1: Microscopic appearance of the germ tubes of the Candida albicans

Fig. 2: Plates showing antifungal susceptibility testing of some antifungal agents used.
CONCLUSION
Following the results obtained, it can be seen that the incidence of candidiasis was found to be high as 126 (63%) among the 200 pregnant women that were sampled. Also, Fluconazole was best in the in-vitro antifungal susceptibility testing of the isolated fungal pathogens (p<0.001) compared with the other agents. The result reveals that Candida albicans was prevalent among the attending pregnant women that patronize Gombe Specialist hospital.

Recommendations
Early diagnosis and prompt treatment of the vaginal candidiasis especially among the risk group in order to avert the complication. Fluconazole in addition to other tested antifungal agents could be used for the treatment of vaginal candidiasis. The use of lubricants (Petroleum jelly, olive oil, etc) during sexual intercourse should be avoided as it increases the risk of contracting the disease. Avoid washing the vaginal area with soap; use water only. Also avoid the use of tight underwear (John, 2000).

REFERENCES


