Resistance Patterns of *Candida Albicans* Isolates to some Antifungals

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Abstract

Vulvovaginal infection affects the female genital organ, unfortunately there is an increasing rate of recurrent vaginal candidiasis owing to microbial resistance with limited data on microbial resistance to antifungal agents in Ghana. This study therefore determined the antifungal resistance patterns of Candida albicans isolates to some antifungals. Three hundred and fifty (350) high vaginal swab (HVS) samples were collected from women who presented with signs and symptoms of VVI. HVS samples were cultured on Sabouraud dextrose agar with 2% chloramphenicol. The polymerase chain reaction was employed to confirm the isolation of C. albicans. Antifungal susceptibility testing was performed and the susceptibility of C. albican isolates to fluconazole, clotrimazole, amphotericin B, nystatin, miconazole and 5' flucytosine were assessed. In the study vaginal infection was most prevalent amongst females in their reproductive age (21 to 30 years). Out of the 350 HVS samples collected, 112 yielded yeast cells with 65 (58%) identified as C. albicans. The C. albicans isolates were resistant to 5' flucytosine (100%), fluconazole (70%), voriconazole (69.2%), miconazole (58.5%) and nystatin (49.2%). C. albicans isolates were more susceptible to amphotericin B (53.8%) and clotrimazole (45.1%), although an appreciable number of the isolates showed some resistance (46.1% and 52.3% respectively). In conclusion, there is a high rate of antifungal resistance with highest resistance rate of 100% (flucytosine) and the least resistance rate of 46.1% (amphotericin B). The various antifungal agents used in the treatment of vaginal candidiasis should be critically considered and appropriate recommendations made to enhance and improve the treatment regimen with these agents.

Keywords: *Candida Albicans*, *Susceptibility Testing, Vulvovaginal infection, Antifungal agents, PCR*

1.0 INTRODUCTION

Candida species cause the most common fungal diseases. There are over twenty species of *Candida* that are pathogenic, but the most isolated pathogenic specie is *Candida albicans* (Center for Disease control, 2016). This species comprises 75% of the fungal species sampled from human specimen. *C. albicans* is one of 200 organisms belonging to the genus *Candida* (Vazquez *et al.,* 2003; Sans-Blas *et al*., 2004).

There have been records of incidence of resistance of microorganisms to antimicrobial agents over the past years (WHO, 2014; Sobel, 2015). These incidence of resistance has resulted in a burden on both health care providers and patients including high cost of treatment and high mortality rates when organisms infects the blood. (WHO, 2014; Patel et al., 2012). A lot of factors have been attributed to the occurrence of microbial resistance, some of which include the misuse of drugs and the inability of patients to complete antimicrobial therapy. It is currently believed that the increasing rate of recurrent VVC (Foxman et al., 2013; Perlin,

2015) is partly due to the development of resistance against antifungal agents.

In Ghana, *C. albicans* has been reported to be the most prevalent *Candida* specie to cause fungal infection (Feglo and Narkwa *et al*., 2012; Abruquah, 2012). High susceptibility of *C. albicans* to fluconazole and voriconazole has also been reported in Ghana (Feglo and Narkwa, 2012), however, reports of resistance only considered antifungal agents as amphotericin B, itraconazole, voriconazole and 5' flucytosine (Feglo and Narkwa, 2012). According to Feglo and Narkwa (2012), voriconazole was the most active antifungal agent with no resistance whereas the overall resistance of the isolates to other antifungal agents ranged from 4.5% to 22.2%, giving a cause for concern (Feglo and Narkwa, 2012).

Abruquah (2012) reported amphotericin B as the most active antifungal agent against vaginal *Candida* isolates with susceptibility rate of 87.2%, though amphotericin B, fluconazole and itraconazole were the only antifungals used in the study (Abruquah, 2012). Reports from Europe and Asia have indicated high resistance rates of *C. albicans* and non-*Candida albicans* to the available antifungals including fluconazole, amphotericin B and voriconazole which has also been a contributing factor to the recurrent infection (Sobel, 2015; Bruna *et al*., 2015; WHO, 2014).

Recurrent vulvovaginal candidiasis (RVVC) is described as contracting 3 or more episodes of vaginal candidiasis in a year. It is a more serious clinical condition due to the recurrence of symptoms, with a strong negative impact on both work and social life (Foxman *et al*., 2013; Sobel, 2015).

Though some reports are available on the resistance pattern of vulvovaginal candidiasis, there are limited current report on the resistance pattern to antifungal agents in Ghana. This study also considered a number of antifungal agents that was previously used in the treatment of vaginal candidiasis and those that are currently used in treatment for the susceptibility testing to show trends of resistance pattern. The study therefore determined the resistance pattern of *C. albicans* to some selected antifungal drugs.

2.0 METHODS AND MATERIALS

2.1. Data and Data Source

Data for this study was obtained through a purposive sampling of 350 women aged between 12 to 80 years with signs and symptoms of vaginal infection between December 2018 and May 2019. Women who did not show signs and symptoms of vaginal infection were excluded from the study. High vaginal swab samples were collected in duplicates with sterile cotton tipped swabs from each participant. The study setting was the Obstetrics and Gynecology department of the Komfo Anokye Teaching Hospital – KATH in the Kumasi Metropolitan Area in Ghana.

2.2. Ethical Approval

Ethical approval was sought and obtained from the Committee of Human Research Publications and Ethics of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology (KNUST) and the Research and Development unit of Komfo Anokye Teaching Hospital, Kumasi, Ghana. Informed consent was also sought from each participant prior to their willful participation. The right of participants to decline participation was strictly emphasized and respected throughout the study data collection.

Data has been and would continually be treated with absolute confidentiality

2.3. Data Analysis

Data cleaning and editing was done daily. The conventional method of polymerase chain reaction (PCR) was employed to confirm the isolation of *C. albicans*. Antifungal susceptibility testing was performed and the susceptibility of *C. albicans* isolates to fluconazole, clotrimazole, amphotericin B, nystatin, miconazole and 5' flucytosine were assessed. Statistical analysis was done using IBM-SPSS version 25.

2.4. Isolation and Maintenance of Yeast

The seal of the sterile cotton swap sample was broken by turning the cap of the swab clockwise, the cotton swab was aseptically inserted into 12 mL sterile nutrient broth in a test tube under the Skan biosafety cabinet. It was quickly stirred in the broth and gently removed by rotating on the walls of the test tube above the level of the volume of broth. The inoculated broth was then incubated at 37°C for 24 h for growth of organisms. After incubation, ten microlitre of culture in broth was aseptically streaked on 20 mL sterile solidified Sabouraud dextrose agar containing 2% w/v chloramphenicol. The inoculated agar plates were then incubated at 37°C for 48 h. Colonies obtained after incubation were transferred into 20% w/v glycerol broth and stored at -80°C until needed (Milan and Zaror, 2004).

2.5. Assay Germ Tube formation

Germ tube formation is a unique characteristic of *C. albicans* and *C. dubliniensis* amongst the other *Candida* species. Yeast cells of *C. albicans* are able to form hyphae with spores with or without constriction (Ha *et al*., 2011). Cells stored in 20% w/v glycerol broth were revived by aseptically transferring 10 µL into 5 mL sterile nutrient broth. The inoculated broth was incubated at 37 °C for 48 h (Milan *et al*., 2002). After 48 h of incubation 10 µL of culture in the nutrient broth was aseptically streaked on a 20 mL sterile solidified Sabouraud dextrose agar (SDA) containing 2% w/v chloramphenicol under the Skan biosafety cabinet and incubated at 37 °C for 48 h. After incubation, a small discrete pure colony of yeast was transferred into sterile tubes containing 0.5 mL human sera. The mixture was incubated at 37 °C for 2 to 3 h. After, a drop was placed on a clean microscope slide and covered with a cover slip. The prepared slide was examined microscopically at a magnification of x10 and x40. The appearance of small or long tube-like filaments projecting from the cell surface confirmed formation of germ tubes which is characteristic of *C. albicans* (Nihad *et al*., 2000). The procedure was carried out in triplicates for all isolates.

2.6. Extraction of DNA from Isolated C. albican Strains

DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB), as described by Doyle and Doyle, (1990) and Dellaporta *et al*. (1983). Small discrete colonies on Sabouraud dextrose agar were dispersed into a 2 mL eppendorf tube with an extraction buffer of 800 µL [2 g CTAB, 2 g PVP, 28 mL NaCl, 4 mL EDTA (p H 8.0), 10 mL Tris – HCl (p H 8.0) and 0.1 mL 2- mercaptoethanol]. The mixture was incubated with intermittent vortexing at 65 ° C for 30 min, to ensure a uniform temperature within the incubated tube. After, it was centrifuged at 14000rpm for 15

min. After centrifugation 600 µL of the supernatant was transferred to a fresh tube with equal volume of phenol – chloroform – isoamyl alcohol. The solution was mixed gently and centrifuged at 14000 rpm for 15 min. Five hundred microlitres (500) µL of the supernatant was again transferred to a fresh tube with 400 µL cold isopropanol at - 20 ° C and gently mixed by inversion. The solution was centrifuged at 14000 rpm for 15 min. The DNA pellets adhered to the tube was visualized. The liquid phase was then released and DNA washed twice with 400 µL of 80% ethanol. The pellet was 161 set to dry for approximately 12 hrs with the tubes inverted upon filter at room temperature. The pellet was resuspended in 200 µL TAE buffer solution plus 5 µL RNAse. The solution was then incubated at 37 ° C for 1 hour and stored at - 20 ° C.

2.7. Determination of the Quality of extracted DNA

The quality of the extracted DNA was determined using the method as described by Kim and Hamada, (2005); Dellaporta *et al*. (1983) and Doyle and Doyle, (1990). 2% agarose gel was 167 prepared with 0.5 µg/mL ethidium bromide. The prepared gel was poured into the cassette of the electrophoretic tank with the electrophoretic comb inserted whiles the tank was adjusted until it was well balanced. The molten gel was allowed to solidify at 25 C after which 1× Tris-acetate-EDTA (TAE) buffer was poured on the solified gel to cover its surface. The combs were gently removed from the gel to create wells. The wells were washed by continuous pipetting in and out of each well to remove gel debris. Ten microlitres each of the extracted genomic DNA was pipetted into 0.5 mL eppendorf tubes. Five (5) microlitres of the loading dye bromophenol blue of concentration 5 mM was added to the 10 µL genomic DNA. The samples were loaded in the wells and allowed to settle. The samples were run at 100 V for 45 min. The resulting bands were photographed with the aid of 176 the UV light and visualized to check the quality of DNA extracted

2.8. PCR Amplification

Polymerase chain reaction (PCR) was used for the amplification of fungal cells specific for fragments of rRNA. The pair of primers (5'TGTTGCTCTCTCGGGGGCGGCCG 3' and 5' AGATCATTATGCCAACATCCTAGGTTAAA 3') were specific for amplifying a fragment of the rRNA gene of *C. albicans* as described by Mannarelli and Kurtzman (1998). Conventional electrophoresis on 2 per cent w / v agarose gel at 100V for 1 h resolved the amplified PCR products.

2.8.1. PCR protocol for Amplification of a 175 Base Pair *C. albicans* special Specification for fragments of rRNA gene

Genomic DNA of the isolates were evaluated using PCR. The PCR amplification targeted the fragments of rRNA genes of C. albicans. Primer sequence CALBF: 5'- TGTTGCTCTCTCGGGGGCGGCCG 3 and CALBR: 5'- AGATCATTATGCCAACATCCTAGGTTAAA -3 were employed for the amplification. The PCR amplifications were performed in a 15 µL reaction mix which consisted of 10 ng of genomic DNA, 3 µL of 5x PCR buffer (mi- red load taq mix, Metabion Int), 0.2 µL each of the forward and reverse primers per manufacturer's protocol (Inquaba Biotec Limited, South Africa). The suspension was adjusted with PCR grade water to

make up the final volume. PCR amplification was performed using the PCR cycling conditions (Table 2.1).

Table 2.1: PCR Time and Temperature

Cycles	Temperature	Time/sec
Initial denaturation	94	30
Denaturation	94	60
Annealing	55	30
Extension	72	60
Final extension	68	300
Hold	4.1	3600

*35 cycles of denaturation, annealing and extension.

2.9. Agarose Gel Electrophoresis of Amplified DNA and Scoring of DNA Fragments

The amplified DNA products were analyzed in 1x Tris acetate acid EDTA (TAE) buffer by running electrophoresis on 2 percent w / v agarose gel at 100 V for 1 hour. After staining with ethidium bromide (0.5 µg / mL) for 1 min, the amplicons were visualized and photographed under ultra violet light (Figure 3.3 and 3.4).

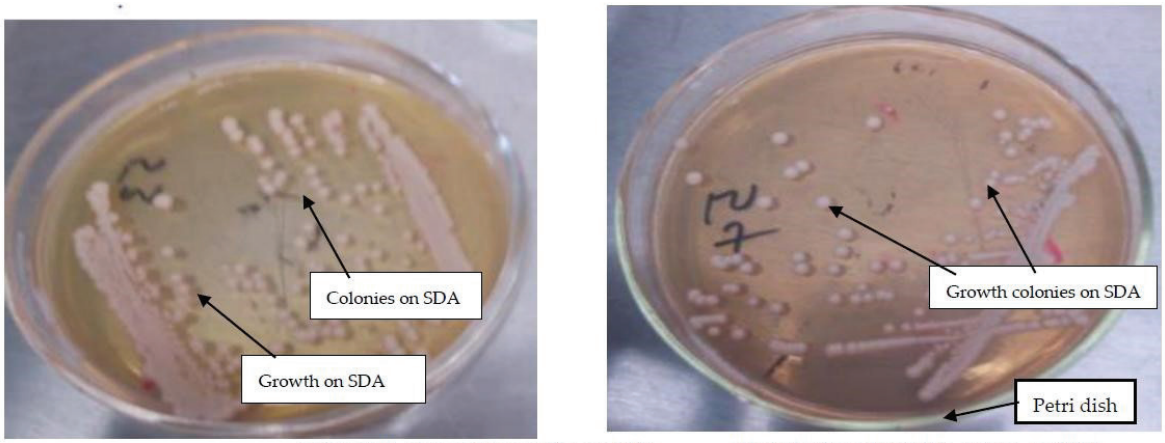
2.10. Antifungal Susceptibility Testing of C. albicans Isolates

Antifungal susceptibility of the *Candida* isolates to amphotericin B (AMB; 20 µg), fluconazole (FCA; 25 µg), nystatin (NYS; 100 units), miconazole (MCL; 10 µg), clotrimazole (CTM; 10 µg), voriconazole (VOR; 1 µg) and 5′ flucytosine (FLC; 1 µg) was assessed using the disc diffusion method (Pfaller and Diekema, 2012; CLSI, 2012). An inoculum size for susceptibility test was standardized to 0.5 McFarland in a calibrated densitometer. Within 10 min after adjusting the turbidity of the inoculum suspension, a sterile cotton tipped swab was dipped in the solution, rotated several times and pressed firmly on the inside wall of the tube above the liquid. The pH of the solutions were neutral. The dipped cotton swap was aseptically inoculated by streaking over the surface of a dried 20 mL Mueller-Hinton agar plate. Streaking was rotated three times to ensure even distribution of the inoculum. The standard antifungal discs were then applied on the surface of the inoculated agar plate using a disc dispenser (Oxoid 6-place, 90 mm) and incubated at 37°C for 18 h. The zones of inhibition for each of the antifungal agent was measured in millimeters using a measuring ruler and compared with breakpoint values according to reference values from CLSI (2012).

3.0 RESULTS

3.1. Identification and Isolation of C. albicans

Out of the three hundred and fifty (350) clinical samples that were cultured on Sabouraud dextrose agar (Figure 3.1). One hundred and twelve (112) were confirmed as yeast cells whereas 74 isolates appeared as germ tubes which is characterized by hyphae production (Figure 3.2). Sixty five (65, 18.5%)) out of the three hundred and fifty samples (350) isolates were confirmed as *C. albicans* (Figure 3.3 and 3.4)



A: Growth of *Candida* species on SDA **B:** Colonies of *Candida species* on SDA

Figure 3.1: Growth pattern of *Candida* species on Sabouraud dextrose agar augmented with 2% chloramphenicol after 48 hours of incubation.

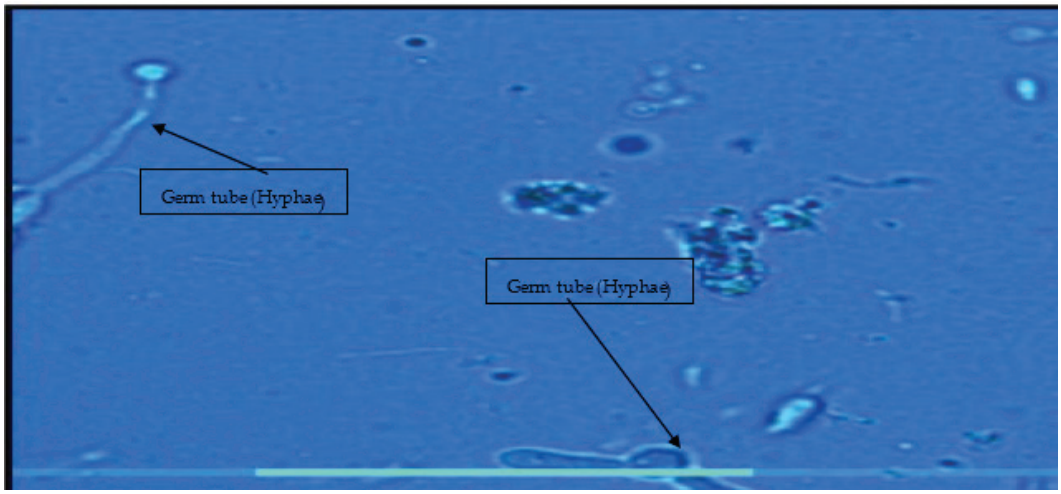


Figure 3.2: ***Germ tube formation after 2 to 3 hours of incubation in 0.5 mL human serum***

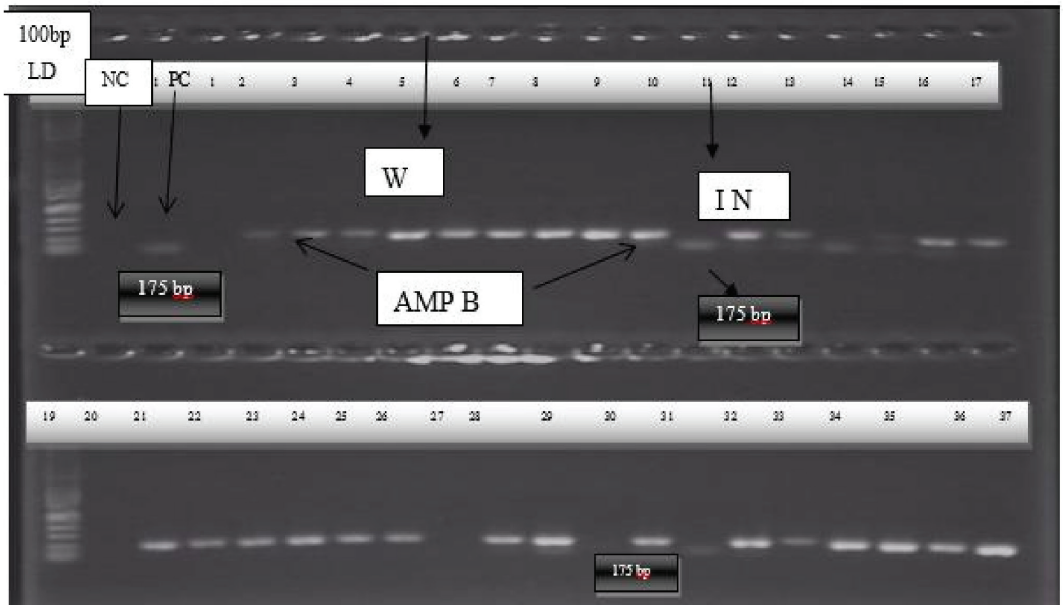


Figure 3.3: ***Gel image showing the 175bp PCR amplicon of the rRNA gene of*** *C. albicans****. LD- 100bp DNA ladder,***

NC- Negative control, PC- Positive control, IN- Isolate numbers 1 – 36; DNA samples of *C. albicans* ***isolates, AMP B- Amplified bands***

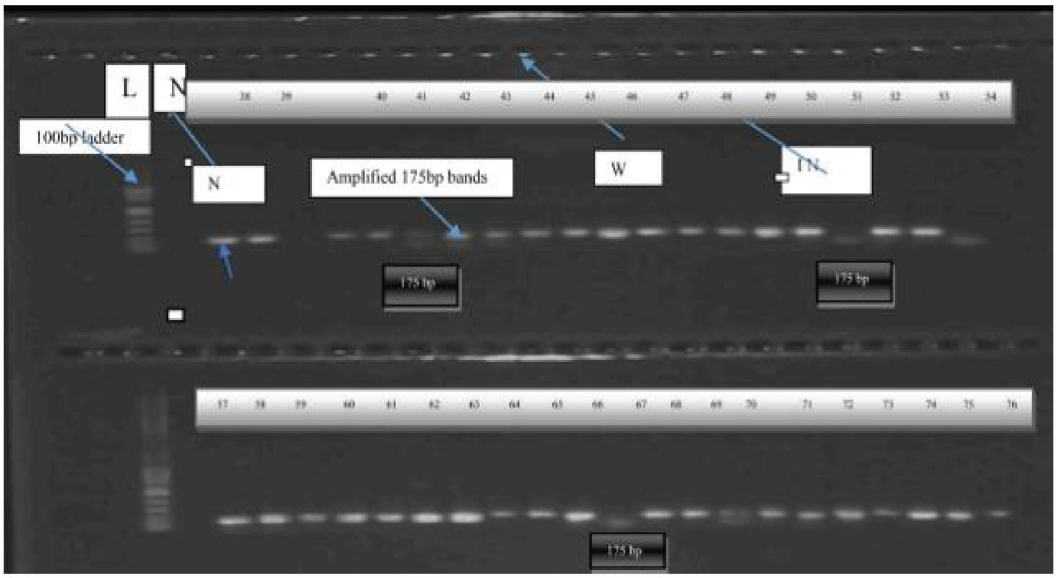


Figure 3.4: ***Gel image showing the 175bp PCR amplicon of the rRNA gene of*** *C. albicans****. L- 100bp DNA ladder, N-***

Negative control, IN-Isolate numbers 37 – 76

3.4. Antifungal Susceptibility Testing

Antifungal susceptibility testing was assessed to determine the resistance and sensitivity pattern of *C. albicans* isolates to the selected antifungal agents by the appearance of clear zones of inhibition.

Susceptibility of *C. albicans* isolates to amphotericin B, fluconazole, nystatin, miconazole, clotrimazole, voriconazole and 5' flucytosine was assessed using the disc diffusion method.

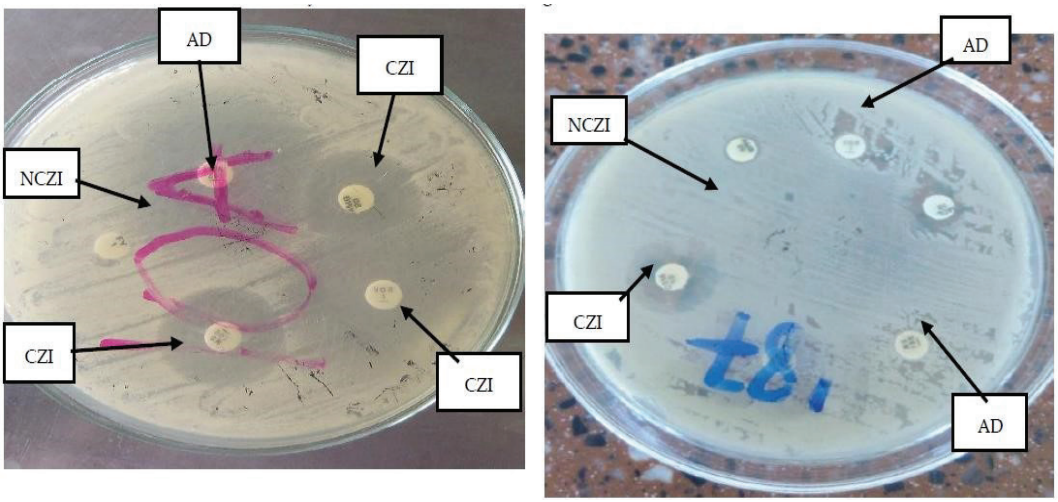


Figure 3.5: ***Zone of inhibition post 24 hours incubation on Mueller-Hinton agar using the disc diffusion method. NCZI; No clear zone of inhibition, CZI; Clear zone of inhibition, AD; Antifungal disc***

3.5 Susceptibility of C. albicans Isolates to Reference Antifungals

The isolated *C. albicans* strains were classified as either susceptible, intermediate or resistant depending on the size of zone of inhibition when compared to the breakpoint values from CLSI M44-A (2012). Resistance rate of the isolates ranged from 46.1% to 96% whiles the susceptibility rate ranged from 10.7% to 53.8%. Isolates were resistant to 5' flucytosine (96.9%) and showed most susceptibility to amphotericin B (53.8%) followed by clotrimazole (46.2%). High resistance of the isolates was also observed against the standard drugs fluconazole (70%), voriconazole (69.2%), miconazole (58.4%), nystatin (49.2%), clotrimazole (52.3%) and amphotericin B (46.1%).

Table 3.2 ***Clinical Breakpoints of Isolates***

	Number (%) of isolates		
Antifungal agent	S	I	R
Fluconazole	16 (24%)	3 (4.6%)	46 (70%)
Amphotericin B	35 (53.8%)	0 (0%)	30 (46.1%)
Nystatin	28 (43.1%)	4 (7.6%)	32 (49.2%)
Voriconazole	19 (29.2%)	1 (1.53%)	45 (69.2%)
Miconazole	7 (10.7%)	20 (30.7%)	38 (58.5%)
Clotrimazole	30 (46.1%)	1 (1.53%)	34 (52.3%)
5' flucytosine	0 (0%)	1 (1.53%)	63 (96.0%)

Zone of Inhibitions showing; ***S: Susceptibility, I: Intermediate and R: Resistant***.

Susceptible: observed zone of inhibition greater than or equal to the size of the standard zone of inhibition.

Intermediate: observed zone of inhibition when concentration of the antifungal drug has an uncertain therapeutic effect.

Resistant: observed zone of inhibition smaller than the standard zone of inhibition.

3.6. Discussion

Over the years, there has been increasing incidence of antimicrobial resistance (WHO, 2014; Patel *et al.,* 2012). This increasing incidence has led to the rising search for new antimicrobial agents against microorganisms. Once microorganisms invade a host, they are able to adapt to the host environment and cause infection. The body's immune system are the first line of defense against microorganisms (Kashid *et al.,* 2011). The ability of the microorganisms to suppress the body's immunity makes it necessary to introduce antimicrobial agents to clear the microorganisms. Antimicrobial agents have the ability to cause cidal and static effect that can inhibit the specific organism (Dharwad and Saldanha, 2011; Ghannoum *et al*., 2016).

This study showed that the prevalence of *C. albicans* causing vaginal infection was high compared with non-*Candida albicans*. Studies carried out on women visiting a gynaecological clinic in Kumasi, Ghana, also reported *C. albicans* to be the most predominant *Candida* species (21%) causing vaginal candidiasis (Abruquah, 2012; Feglo and Narkwa, 2012). Out of the total 112 yeast cells isolated in this study, 58% of the yeast cells were detected to be *C. albicans*. Sobel (2015), Fidel (2000) and Feglo (2012) reported *Candida albicans* as the most prevalent yeast cell that causes vaginal infection.

The standard antifungal drugs employed for this study were the azoles; fluconazole, voriconazole, miconazole and clotrimazole, the polyenes; amphotericin B and nystatin and pyrimidine analogues; 5 flucytosine. These antifungal drugs have over the years been used in the treatment of Candida infections.

There was a high rate of resistance of *C. albicans* to the antifungal drug 5' Flucytosine (96.9%) (Table 3.2), this could be as a result of the Fungistatic effect of flucytosine to *C. albicans*. Though 5-FC has no intrinsic antifungal capacity, however when taken, its metabolites become incorporated into DNA and RNA molecules of fungal cells (Vandeputte *et al*., 2012; Vermes, 2000). 5-FC enters fungal cells and is converted to 5-fluorouracil (5-FU), and then into 5-fluorouridylic monophosphate (FUMP). This pyrimidine analog is then incorporated into RNA, resulting in disruption of protein synthesis. Two basic mechanisms of resistance were described by Vermes (2000) and Peman (2009) which includes deficiency in the enzymes necessary for cellular transport, uptake of 5-FC and its metabolism and the second mechanism is the increased synthesis of pyrimidines, which compete with 5-FC during RNA synthesis, thus diminishing its ability to affect RNA synthesis (Vermes, 2000; Peman *et al*., 2009).

The azole derivative antifungal drugs used in this study were fluconazole, voriconazole and miconazole (Table 3.2; Fig. 3.5). These antifungal drugs targets the fungal cytochrome P450 enzyme lanosterol 14α-demethylase (Flowers *et al.,* 2014). According to this study, there was a high resistance rate of *C. albicans* to fluconazole (70.8%), voriconazole (69.3%) and miconazole (58.5%). These antifungal agents are known for their fungistatic effect on fungal cells. Resistance mechanisms for azoles include changes in the 14α-demethylase gene, changes in the pathway of sterol synthesis, reduction or overexpression of the target enzyme, and increased activity and number of drug efflux pumps (Flowers *et al*., 2012; Peman *et al*., 2009).

The polyenes antifungal drugs used in this study were amphotericin B and nystatin. Before azoles were developed, they were the antifungal therapy medications for systemic fungal infections. Polyene interacts with fungal membrane sterols, leading to the production of

aqueous membrane pores (Gray *et al*., 2012). The development of these aqueous pores in the cell membranes leads to alterations in membrane permeability, which causes several problems including leakage of ions, a property that leads to cell death. In the study, resistance rate of amphotericin B and nystatin was 46.2% and 49.3% respectively. Amphotericin B recorded the lowest resistant rate to the fungal cells, this could be as a result of the Polyenes fungicidal effect on fungal cells.Resistance to polyene drugs such as amphotericin B and nystatin is rare with a suggestion that development of resistance occurs by selection of naturally occurring resistant cells present within the population (Herbrech *et al.,* 2002; Ghannoum *et al*., 1999). Resistance of fungal cells to these antifungal agents involves alteration of the sterol synthesis pathway, thereby limiting the amount of sterols present in the cell membrane, leading to resistance to polyene type of antifungals (Butts and Krysan, 2012).

Resistance rate of fungal cells to clotrimazole was 52.4%. Though clotrimazole is known as one of the standard antifungal drugs used in the treatment of vulvovaginal candidiasis, it has a fungistatic effect on fungal cells and as an azole derivative resistance include changes in the pathway of sterol synthesis, reduction or overexpression of the target enzyme, and increased activity and number of drug efflux pumps (Flowers *et al*., 2012; Peman *et al*., 2009).

According to Abruquah (2012), susceptibility of the isolates to antifungal agents ranged from 66.7% to 87.2 for amphotericin B, fluconazole and itraconazole. *C. albicans* were more susceptible to amphotericin B with sensitivity of 87.2%. This observation is also in line with results of the present study as amphotericin B (53.8%) was more active against the isolated species, though there was an appreciable decline in sensitvity of the isolates to amphotericin B which could be due to more resistance genes strains of *Candida albicans*.

Another study in Ghana reported a significant increase in resistance of isolates to commonly used antifungal agents (itraconazole, fluconazole, ketoconazole and nystatin) (Siakwa *et al.,* 2014). The current findings from this study are consistent with that of Siakwa *et al.* (2014) with amphotericin B and clotrimazole showing susceptibility results of 35 (53.8%) and 30 (45.1%) respectively (Table 4.13). Most of the participants were in their reproductive ages between 21 to 35 years (63%) and 36 to 45 years (24.9%). This is consistent with most findings which reported that VVC mostly occurs in 60 to 75% of females in their reproductive age (Babin *et al*., 2013; Shilpa and Swati, 2016) and that sexually active women are more prone to vaginal infection (Das Neves *et al.,* 2011; De Araujo *et al*., 2011; Spiegel, 1991).

Out of the 350 samples collected, 65 (18.5%) isolates were confirmed as *C. albicans*. In a study carried out on women visiting a gynaecological clinic in Kumasi, Ghana, *C. albicans* was reported to be the most predominant *Candida* species (21%) causing vaginal candidiasis (Abruquah, 2012; Feglo 353 and Narkwa, 2012). This is consistent with the findings of this study since *C. albicans* was the most common (58%) organism amongst the yeast cells isolated. Another study in Ghana reported a significant increase in resistance of isolates to commonly used antifungal agents (itraconazole, fluconazole, ketoconazole and nystatin) (Siakwa *et al.,* 2014). The current findings from this study are consistent with that of Siakwa *et al.* (2014) with amphotericin B and clotrimazole showing susceptibility results of 35 (53.8%) and 30 (45.1%) respectively.

4. CONCLUSION

The *C. albicans* isolates were resistant to 5' flucytosine (100%), fluconazole (70%), voriconazole (69.2%), miconazole (58.5%) and nystatin (49.2%). *C. albicans* isolates were more susceptible

to amphotericin B (53.8%) and clotrimazole (45.1%), although an appreciable number of the isolates showed some resistance. The treatment of any vaginal infection must be based on laboratory investigations to identify the causative agents and administer the right medication.

The use of amphotericin B, fluconazole, miconazole and clotrimazole in the treatment of vaginal candidiasis should be further assessed based on the level of resistance of *C. albicans* to these medications. Further studies on antifungal susceptibility of *C. albicans* isolated from larger sample size testing should be frequently carried out to monitor resistance of *C. albicans* to the currently used antifungals. The use of the various antifungal agents used in the treatment of vaginal candidiasis should be critically considered and appropriate recommendations made to enhance and improve the treatment regimen with these agents.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS CONTRIBUTIONS

AKS conceptualized and designed the methodology of the study. AKS acquired the data and was part of the team who wrote the literature review, both theoretical and empirical as well as parts of the discussion of the results. AKS, SAS, CA, VEB and YDB analyzed the data wrote some parts of the discussions of the manuscript. SAS, CA, VEB and YDB critiqued the work and provided some theoretical underpinnings to the study. AKS, SAS, CA, VEB and YDB proof read the paper thoroughly and corrected the manuscript linguistically. All authors agree to be accountable for all aspects of the work and jointly own the work. All authors read and approved the final manuscript.

COMPLIANCE WITH ETHICAL GUIDELINES

In addition to ethical approvals, written consent was also sought from the management of Komfo Anokye Teaching Hospital Microbiology Department and Medilab Diagnostics. The aim and benefits of the study were clearly explained to the participants. The right of participants to decline participation was strictly emphasized and respected throughout the study data collection. Data has been and would continually be treated with absolute confidentiality.

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