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Prevalence and Antifungal Susceptibility Patterns of Yeast Isolates at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana

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Research Article

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ABSTRACT

Aims: To determine the prevalence of yeast species and their antifungal susceptibility profiles at Komfo Anokye Teaching Hospital (KATH), Kumasi.
Study Design: Cross-sectional design.
Methodology: This study was conducted in 2009 at the bacteriology laboratory at KATH, Kumasi, Ghana. In six-months, 528 clinical samples comprising 186(35%) high vaginal swabs, 109(21%) cerebrospinal fluid, 127(24%) urine and 106(20%) sputum were cultured on sabouraud dextrose agar. Yeast growths were identified by their characteristics, indian-ink staining and germ tube test and then confirmed with the API ID 32 C test kits. Antifungal susceptibility tests were performed using ATB™ FUNGUS 3 test kit.
Results: Out of 528 samples tested 67 yielded yeasts giving a prevalence of 12.7%. *Candida albicans* was the commonest species isolated with a prevalence of 33(49.3%) followed by *Candida glabrata* 12(17.9%), *Candida tropicalis* 8(11.9%), *Candida dubliniensis* 4(6%), *Candida krusei* 3(4.5%) and *Candida sake* 2(3%), whilst *Candida guilliermondii* and *Candida parapsilosis* prevalence was 1(1.5%) each and *Cryptococcus neoformans* prevalence was 3(4.5%). All the isolates were susceptible to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole except *C. albicans, C. glabrata, C. tropicalis and C. krusei* all having about 79% susceptibility to flucytosine, amphotericin B, and itraconazole (MICs 0.125-8mg/l). Voriconazole was the only agent to which no resistant yeast was detected. All the *Candida krusei* isolated were resistant to fluconazole (MICs ≥ 64mg/l). Generally yeast resistance ranges from 4.5% to 22.2% to flucytosine, amphotericin B, fluconazole and itraconazole.
Conclusion: There were many yeast species isolated, but *Candida albicans* was the most common isolate obtained from all the clinical samples tested except cerebrospinal fluid from which *Cryptococcus neoformans* was the commonest. The overall resistance levels of

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the isolates ranged from 4.5% to 22.2% to flucytosine, amphotericin B, fluconazole and itraconazole. No resistant strains were detected against voriconazole. This high level of resistance (22.4%) in Ghana calls for further investigation. This is the first report on the yeast types and their antifungal susceptibility patterns in Ghana.

Keywords: Yeast infection; antifungal drugs; Candida; Cryptococcus.

1. INTRODUCTION

Yeasts are common fungal agents affecting humans. They cause diseases with severity ranging from benign to potentially life-threatening infections, with the most common yeasts being the *Candida* species and *Cryptococcus* species (Pfaller and Diekema, 2007). *Candida albicans* remains the predominant species causing over half of all the yeast infection cases in the world (Pfaller and Diekema, 2007). Increase in the prevalence of yeast infections caused by non-albican *Candida* such as *Candida glabrata, Candida krusei, Candida tropicalis and Candida parapsilosis* have been reported in many parts of the world (Enwuru *et al*., 2008). *Cryptococcus neoformans* is the major species of the Cryptococcus that infect humans, but other species such as *Cryptococcus albidus* and *Cryptococcus laurentii*, have also been reported to cause human infections (Kordossis *et al*., 1998). In Ghana fungal infections are common, but current scientific data on their prevalence and antifungal susceptibility are scanty. Ghanaian women get vaginal yeast infections accompanied by vaginal itching, burning and discharge, supporting the report that about 24.4% of Ghanaian women report to the clinic with Candida infection (Deceuninck *et al*., 2000). Dermatophyte infections are also common in Ghana as was reported in a study where 105 (23.1%) of 454 school children had tinea capitis (Hogewoning et al., 2006). Another study also reported that 10.6% of fungal infections to be the cause of other skin infections (Doe *et al*., 2001).

Antifungal agents commonly used to treat yeast infections include flucytosine, fluconazole, amphotericin B, voriconazole, clotrimazole, nystatin, capsofungin and ketoconazole. The problem with the use of antifungal agents, apart from safety and cost is the development of drug resistant strains (Pfaller *et al*., 2005). The development of antifungal resistance among yeasts has been linked to misuse and inappropriate prescription of antifungal agents (Pfaller *et al*., 2005). Meanwhile community onset of yeast infections including candidemia is not uncommon and appears to be increasing worldwide due to changing health care practices (Pfaller *et al*., 2011c). In Ghana, antifungal drugs are sold over the counter, a practice which encourages self medication and therefore may contribute to the development and spread of antifungal resistance. Meanwhile current information or data relating to yeast types and their susceptibility patterns to antifungal agents at KATH is scanty. In addition, there is no National Surveillance Programme to monitor antifungal resistance among yeasts and other pathogenic fungi in Ghana.

The aim of this study was to determine the prevalence of yeasts types and their antifungal susceptibility patterns of yeast isolates obtained from patients attending Komfo Anokye Teaching Hospital.

2. MATERIALS AND METHODS

The study was conducted at the microbiology laboratory of KATH from January to June 2009 after obtaining ethical clearance. The informed consent was obtained from the patients after the purpose of the study was explained to them. Samples of those who objected were not included in the study. Non-repeated 186 samples of High vaginal swab (HVS), 127 urine, 109 cerebrospinal fluid (CSF) and 106 sputum samples were analyzed. The HVS samples were taken from female patients whose ages ranged from 10 years to 61 years and who reported at the Out-Patients Department (OPD) with complaints of vulvovaginitis and/or vaginal discharge. The sputum samples were collected from both male and female patients with chronic cough and chest pains but have tested negative for tuberculosis. There were 55 males and 51 females whose ages ranged from 9 years to 76 years. Those patients on admission in the hospital were 41 whilst 61 patients were from the community and reported at the OPD and were referred to the bacteriology laboratory for diagnostic purposes. Urine samples were obtained from 51 males and 76 females. Their ages ranged from 12 years to 78 years. These were patients with complaints of frequency of micturiction, painful micturiction or urine retention and were suspected of urinary tract infection. Those patients with indwelling catheter were 49 and were on admission on the wards. These patients with indwelling catheter had their samples collected from the wards and submitted to the laboratory. CSF samples were collected from individuals with convulsion, stiff neck and headache suspected of meningitis. These patients were mostly children under 10 years though other ages were also included. Ambulant patients came to the laboratory with their test request forms and the samples were collected. Bed-ridden patients had their samples collected on the ward and then brought to the laboratory by a ward assistant.

2.1. Analysis of Samples

2.1.1 Analysis of Urine

Fresh mid-stream urine samples were collected from patients suspected of urinary tract infections and who were referred to the laboratory. The patients were given clean dry sterile urine culture bottles for the samples. The patients were told to allow first potion of urine to flow away, and then to collect the “mid stream” of the urine directly into the bottle, replace the lid firmly on the bottle and then return the specimen as soon as possible to the bacteriology laboratory. The urine samples were labeled with the patient’s number, age and sex. At the laboratory, the urine sample was transferred into clean, dry sterile test tube and then centrifuged at a speed of 3000rpm for 5 minutes. After centrifugation, the supernatant was decanted and the sediment was streaked onto sabouraud dextrose agar using a loop calibrated (0.01ml) to deliver a constant amount of urine. A wet film was then prepared and another one was dried and stained by Grams method and Indian ink, and then observed for yeast cells.

2.1.2 Analysis of HVS

The HVS samples were collected from female patients who complained about gynaecological problems such as vaginal discharge or vulvovaginitis and were referred to the laboratory for diagnostic purposes. High vaginal swabs were taken from them by trained female medical assistants at the laboratory using sterilize cotton-tipped swabs. The swabs were labeled with the patient’s number, age and then transported in Stuart transport medium to the laboratory. The swab was used to inoculate the SDA and then spread by streaking

using a sterile loop. The swab was replaced in its container, shaken vigorously and then discarded. After centrifugation, the supernatant was decanted and a wet film was prepared from the sediment, and then observed for yeast cells. The remaining sediment was used to prepare a smear for Gram and Indian ink staining. The stained slides were examined under the microscope for budding yeast cells and pseudohyphae.

2.1.3 Analysis of Sputum

Patients with cough who were referred to the laboratory were asked to cough up sputum and to deposit it directly into a sterile wide-mouth monowax container. The patients were instructed go home and to collect the sputum specimen the following day. The specimen must be collected first in the morning when they got up. They were told to provide deep coughed specimen. Upon arrival in the laboratory the specimens were visually examined. Those that were watery were considered to be saliva and were rejected. Those that were mucopurulent and frothy were accepted. The accepted samples were labeled with the patient's number, age and sex. Using a sterile microbiological inoculating loop, a mucopurulent part of the sputum specimen was picked and inoculated onto SDA.

Smears were prepared for a wet film, Indian ink and Gram stain and then examined under the microscope for budding yeasts and pseudohyphae.

2.1.4 Analysis of CSF

CSF samples were taken by physicians and placed into sterile bijoux bottles and submitted to the laboratory. Cerebrospinal fluid was collected by medical officers via lumbar puncture and submitted to the laboratory by ward assistants. The samples were labeled with the name, age and sex. A loopful of CSF was inoculated on SDA plates. Smears for Gram and Indian ink staining were prepared from the remaining CSF sample. The stained slides were examined under the microscope for budding yeast cells and pseudohyphae.

Preliminary identification of the yeasts involved Gram staining, Indian ink staining and wet-film microscopy directly on the specimen. The detection of more than five leukocytes per oil immersion field and the detection of more than five yeast cells with pseudohyphae in the gram stained smear were considered to be an infection for HVS. The detection of any five yeast cells and leukocytes were considered to be an infection for urine and sputum but any yeast cell in addition to five or more leukocytes detected in the CSF was considered to be an infection. The samples that were considered to be infected were then inoculated onto sabouraud dextrose agar (SDA, Oxoid Ltd, Basingstoke Hants, England) and incubated at room temperature for a week but inspected for growth daily. When no growth occurred after 48 hours the plates were re-incubated for one week, but inspected for growth daily. When growth occurred the growth characteristics involving the colonial morphology, colour and pigment formation. Identical colony types were considered as one yeast type and were harvested and stored in 20% glycerol broth at -18^{o}C for further identification later. The stored isolates were later subcultured onto sabouraud dextrose agar to obtain pure culture which was used for further analysis. The growths from the purity plates were identified to the species level using the germ tube test and the mini API ID 32 C test kit (bioMerieux, Marcy-l'Etoile, France). The yeast susceptibility tests were performed using the ATB FUNGUS 3 test kit (bioMerieux, Marcy-l'Etoile, France) against flucytosine (5-FC), amphotericin B (AMB), fluconazole (FCA), itraconazole (ITR) and voriconazole (VRC).

2.2 In Vitro Antifungal Susceptibility Testing of Yeasts

Antifungal susceptibility was performed using the ATB™ FUNGUS 3 strip. The strip consists of 16 pairs of cupules. The first pair cupules did not contain any antifungal agent and were used as positive growth controls. The next 15 pairs contained the antifungal agents to be tested at several concentrations as shown in Table1 below.

Table 1. Range of concentrations of antifungal agents tested

Antifungal agents	*Range of concentration (mg/l)
Flucytosine	4 &16
Amphotericin B	0.5, 1, 2, 4, 8 & 16
Fluconazole	1, 2, 4, 8, 16, 32, 64 & 128
Itraconazole	0.125, 0.25, 1, 2 & 4
Voriconazole	0.06, 0.125, 0.25, 0.5, 1, 2, 4 & 8

**Antifungal concentrations on the ATB Fungal 3 test strip*

2.3 Preparation of Yeast Inoculum for the Antifungal Susceptibility Tests

Using a sterile inoculating loop several similar yeast colonies on the SDA agar plate were transferred into 2ml of API® NaCl 0.85% medium (provided with the kit) to form a suspension of turbidity equivalent to 2McFarland, which was measured by a Densimat (included in the kit pack). After mixing 20µl of the suspension was transferred into 7 ml of ATB F2 medium and used for the inoculation into the ATB FUNGUS 3 strips.

2.4 Inoculation of the ATB Fungus 3 Strips

The ATB F2 medium containing the organism was homogenized and 135µl of the suspension was dispensed into each of the 16 cupules on the strip. The strips were then incubated at 37℃ for 24 and inspected for growth, when growth pattern was not clear the strip was re-incubated for another 24 hours.

2.5 Minimum Inhibitory Concentration (MIC) Determination

After observing and comparing the growths in the test cupules with the growth in the control cupules, the degree of growth in each cupule was quantified visually and then scored as in table 2.

Table 2. Growth scores and their interpretations

Definition	Score
No reduction in growth	4
Slight reduction in growth	3
Distinct reduction in growth	2
Very weak growth	1
No growth	0

The MIC for amphotericin B was the lowest concentration with a score of 0 where there was no growth. The MICs for fluconazole, itraconazole and voriconazole were the lowest concentrations with a score where there were distinct reductions in growth. Flucytosine which was tested at two concentrations of 4mg/l and 16mg/l had the growth scores and their interpretations as follows:

When the score at the concentration of 4mg/l is 0, 1 or 2 and that at the concentration of 16mg/l is 0, 1 or 2 the organism is considered as susceptible to flucytosine at MIC of 4mg/l. When the growth score at 4mg/l is either 3 or 4 and that at the concentration 16mg/l is reduced to 0, 1 or 2 the organism is considered to be in the intermediate category. When the growth score at the concentration of 4mg/l is either 3 or 4 and that at the concentration of 16mg/l is also 3 or 4, then the organism is considered to be resistant to flucytosine.

The CLSI interpretive breakpoints Susceptible (S), Intermediate susceptible (I) and Resistant (R) were adopted as follows: FCA S≤ 8mg/l; I: 16-32mg/l and R≥ 64mg/l for *Candida* species and S≤ 4mg/l; I: = 8mg/l and R≥ 16mg/l for *Cryptococcus neoformans*; ITR (S≤ 0.125mg/l; I: 0.25-0.5mg/l and R≥ 1mg/l for Candida species and VRC S≤ 1mg/l; I: =2mg/l and R:≥ 4mg/l for *Candida* species using *Candida parapsilosis* (NCTC 3104) for quality control, when all attempts to obtain ATTC 22019 failed. Though new breakpoints have been published (Pfaller *et al*., 2011a; Pfaller *et al*., 2011b), these breakpoints are in micrograms. The ATB™ FUNGUS 3 strip used in this study had the drug concentrations in milligrams so the adjusted 24-h clinical break points (susceptible, ≤ 0.125 µg/mL; intermediate, 0.25-0.5 µg/mL; resistant, ≥ 1 µg/mL) for voriconazole against *C. albicans, C. tropicalis*, and *C. parapsilosis* (Pfaller *et al*., 2011a; Pfaller *et al*., 2011b) could not be adopted. Instead the interpretive breakpoints for itraconazole and voriconazole against *Cryptococcus neoformans* considering a previous pharmacokinetic studies breakpoint of 1mg/l suggested by Sheehan et al, (1999) was adopted. Interpretive breakpoints for AMB against *Candida* and *Cryptococcus neoformans* are also not available CLSI (2000), so MIC ≤ 1mg/l as suggested by Sheehan et al. (1999) was applied.

3. RESULTS

A total of 528 samples were analyzed. Out of this number 67 yeast isolates were obtained giving yeast prevalence of 12.7% among the clinical samples. There were 39(186) of the HVS samples that yielded yeasts giving HVS yeast prevalence of about 21%. Yeast prevalence in urine was 14(127), sputum 10(106) and CSF 4(109). Of the 67 yeast isolates obtained, 49.3% were Candida albicans, 17.9% were *Candida glabrata*, 12% were *Candida tropicalis* and 6% were *Candida dubliniensis*. Other yeast species identified were *Candida krusei* (4.5%), *Candida sake* (3%), *Candida parapsilosis* (1.5%), *Candida guilliermondii* (1.5%) and *Cryptococcus neoformans* (4.5%). The distribution of yeast among the sample types analyzed are as shown in Table 3.

Antifungal susceptibility test results indicate that 97% of the yeast isolates were susceptible to voriconazole with 2 out of 33 isolates being intermediate susceptible with no resistant strains detected. Various resistant levels were detected against other antifungal drugs but to fluconazole all isolates of Candida krusei were found to be resistant. Susceptibility levels to other drugs indicate that 83.6% of the yeasts were susceptible to flucytosine, 77.6% to amphotericin B, 71.6% to fluconazole, and 70.1% to itraconazole. Susceptibility test results indicate that 82.1% of the isolates were susceptible to amphotericin B while 80.6% were susceptible to flucytosine. The details of the antifungal susceptibility test results are as shown in Table 4 below.

Table 3: Yeast species isolated from clinical samples tested

Yeast isolate	Number (%) of yeast species isolated from sample type				
	HVS	Urine	Sputum	CSF	Total
Candida albicans	19(48.7)	6(42.9)	7(70.0)	1 (25.0)	33(49.3)
Candida glabrata	7(17.9)	5(35.7)	0(0.0)	0(0.0)	12(17.9)
Candida tropicalis	4(10.3)	2(14.3)	2(20.0)	0(0.0)	8(11.9)
Candida krusei	2(5.1)	0(0.0)	1(10.0)	0(0.0)	3(4.5)
Candida parapsilosis	1(2.6)	0(0.0)	0(0.0)	0(0.0)	1(1.5)
Candida dubliniensis	4(10.3)	0(0.0)	0(0.0)	0(0.0)	4(6.0)
Candida sake	2(5.1)	0(0.0)	0(0.0)	0(0.0)	2(3.0)
Candida guilliermondii	0(0.0)	1(7.1)	0(0.0)	0(0.0)	1(1.5)
Cryptococcus neoformans	0(0.0)	0(0.0)	0(0.0)	3 (75.0)	3(4.5)
Total	39(00)	14(00)	10(00)	4(100)	67(100%)

HVS= High vaginal swab; CSF= Cerebrospinal fluid; N= Number of species isolated; (%) = Percentage isolate in a sample type.

4. DISCUSSION

Yeast infections of the vagina are common problems that cause significant morbidity and affect the well being of women. This study showed yeast prevalence of 21% from high vaginal swabs (HVS) amongst the patients studied at KATH. Vaginal yeast prevalence among the patients is common as the organism easily colonizes mucous membranes (Moyes and Naglik, 2011) such as the vagina, urinary tract and the oral cavity. From these sites organisms multiply to cause symptomatic infection when the physiologic conditions of the site is altered (Kauffman *et al*., 2011). In this current study initial microscopy with wet film was used to assess whether there were just a few yeast cells, which may be considered a mere colonization. On the other hand when large numbers of yeasts (more than 5 yeast cells per film) were seen with pseudohyphae then the diagnosis of yeast infection is established. The microscopy was also used to rule out other infections as follows: the wet film ruled out *Trichomonas vaginalis*, Gram stain ruled out *Neisseria gonorhoeae* and *Gardnerella vaginalis* infections. The Indian ink was used to establish the presence of yeasts leading to the detection of the prevalence of 21% of vaginal yeast infection, but not colonization in this present study. In Nigeria 40.6% prevalence was reported by (Enweani *et al*., 2001), a value much higher than the 21% observed in this study. The difference is probably due to the differences in methodology where microscopy was initially used to screen the patients rather than subjecting every sample to culture.

Table 4. Antifungal susceptibility test results of yeast isolates

Yeast species isolated	No. of isolates	5-FC			AMB		FCA			ITR			VRC		
		S	I	R	S	R	S	I	R	S	I	R	S	I	R
Candida albicans	33	26	4	3	24	9	27	6	0	26	5	2	31	2	0
Candida glabrata	12	11	1	0	11	1	3	9	0	1	8	3	12	0	0
Candida tropicalis	8	6	2	0	5	3	7	1	0	7	1	0	8	0	0
Candida krusei	3	2	0	1	1	2	0	0	3	2	1	0	3	0	0
Candida dubliniensis	4	4	0	0	4	0	4	0	0	4	0	0	4	0	0
Candida sake	2	2	0	0	2	0	2	0	0	2	0	0	2	0	0
Candida parapsilosis	1	1	0	0	1	0	1	0	0	1	0	0	1	0	0
Candida guilliermondii	1	1	0	0	1	0	1	0	0	1	0	0	1	0	0
Cryptococcus neoformans	3	3	0	0	3	0	3	0	0	3	0	0	3	0	0
Total	67	56	7	4	52	15	48	16	3	47	15	5	65	2	0
% Susceptibility		83.6	10.4	6.0	77.6	22.4	71.6	23.9	4.5	70.1	22.4	7.5	97.0	3.0	0.0

S=number sensitive; I=number intermediate susceptible; R= number resistant. 5-FC=flucytocine; AMB=amphotericin B; FCA=fluconazole; ITR=itraconazole; VRC=voriconazole

The results here, however, are consistent with 20.6% reported in Costa Rica (Gross *et al.*, 2007) and 20.15% reported in Brazil (Passos *et al.*, 2005) where initial screenings by microscopy were performed. Also *Candida albicans* was the most frequent yeast followed by *Candida glabrata* isolated from HVS in our study. Similar result was obtained in Libya (Ellabib & ElJariny, 2001) where *Candida albicans* was the dominant isolate. In Nigeria, *Candida kefyr* and *Candida stellatoidea* were reported as the second and third most common species causes of vulvovaginitis respectively, only after *Candida albicans* (Enweani *et al.*, 2001). These findings are in support of our current study as Candida albicans was found as the dominant isolate.

The prevalence of candiduria detected in this study was 11%, but 5.1% was reported in 2003 in Accra, Ghana (Ayeh-Kumi *et al.*, 2007) and 44.4% in Brazil (Passos *et al.*, 2005) among patients in intensive care units (ICU). In all cases in Ghana and Brazil including this current study the commonest yeast isolated from urine was *Candida albicans* followed by *Candida glabrata* and *Candida tropicalis*. All these isolates are known to cause infections even systemic or invasive infection (Chi *et al.*, 2011). The finding of *candida* in the urine of a patient with or without symptoms should not be dismissed. It should not be treated hastily either (Kauffman *et al.*, 2011) because there may be very serious underlying conditions such as Candida pyelonephritis, cystitis, prostatitis, or epididymo-orchitis (Kauffman *et al.*, 2011). Symptoms for the above conditions are little different from those infections produced by other pathogens. In addition Candiduria occurring in critically ill patients should initially be regarded as a marker for the possibility of invasive candidiasis. However it is prudent to test for true infection rather than colonisation before therapy is initiated (Fisher, 2011), to avoid misuse of the drug and the resultant potential for the organism to develop resistance.

The prevalence of yeast in sputum was 9.4%, but as high as 63% was reported in South Africa among HIV negative patients (Patel *et al.*, 2006). In Thailand and Cambodia 48% was reported among HIV- infected patients (Richter *et al.*, 2005) and 34.2% was reported in Nigeria (Enwuru *et al.*, 2008) among HIV-positive patients. It is not clear what accounted for these large differences in prevalence in the three different places. Higher prevalence of yeast among HIV-positive patients was rather expected (Chen *et al.*, 2000) because yeasts cause opportunistic infections usually amongst the immunocompromised individuals, which HIV-positive people usually are. The low prevalence in this present study can be explained by the screening method adopted in which individuals failing to satisfy the screening requirement were rejected in this study. The results are however consistent with reports from Italy (Asticcioli *et al.*, 2009) and (Baran *et al.*, 2000) in which *Candida albicans* was the commonest yeasts isolated followed by *Candida tropicalis* and *Candida krusei*. *Candida albicans* seems to be the commonest yeast type obtainable from many sample types except CSF. This observation is supported by another study elsewhere, in which it was reported that from 1,354 episodes of bloodstream yeast infections *Candida albicans* (48.4%) was the commonest, followed by *C. glabrata* (18.2%), *C. parapsilosis* (17.1%), *C. tropicalis* (10.6%), and then 2.0% was reported for *C. krusei* (Pfaller *et al.*, 2011c).

The prevalence of yeasts isolated from CSF in this study was 3.6% but 2.2% was reported in New Zealand (Chen *et al.*, 2000). Three out of the four yeasts isolated were C*ryptococcus neoformans* and one was identified to be *Candida albicans* supporting views that *Cryptococcus neoformans* was the commonly yeast isolated from cerebrospinal fluid (Chen *et al.*, 2000; Pfaller & Diekema, 2007). It was reported recently that the API identification system has many discrepancies as compared with the PCR (Liguori *et al.*, 2010), but the API system performed better than the VITEK2 and RYIP identification systems, so it was only the PCR method which was superior to the API identification (Liguori *et al.*, 2010). Again

advanced systems involving electrphoretic kayrotyping and the restriction fragment length polymorphism based on the use of 27A probe, and also the use of RT-PCR used to evaluate expression of *CDR1*, *CDR2* and *MDR1* genes have been described (Gallè *et al*., 2011). These methods require expensive equipment and reagents which researchers in resource constrained countries like Ghana find difficult to obtain. Nonetheless, using the mini API ID 32 C test kit (bioMerieux, Marcy-l'Etoile, France) is adequate and it is second only to the molecular techniques (Liguori *et al*., 2010).

A study involving 56 yeast isolates obtained from women with candidal vaginitis in Brazil had no resistant strains to fluconazole (Ribeiro *et al*., 2000). In this present study 12 *Candida glabrata* were isolated out of which 9(75%) were found to be intermediate susceptible or dose dependent to fluconazole, but the remaining was sensitive, and none was resistant. This result is completely different from the results of a study in the USA where as much as 15% of Candida glabrata isolates were found to be completely resistant (Pfaller *et al*., 1999). Another study found 57% *Candida glabrata* isolates in the USA (Richter *et al*., 2005) to be in the intermediate category with 15.2% of them being completely resistant, but in this present study no resistant strains of *Candida glabrata* were found against fluconazole.

Amphotericin B in combination with flucytosine is the drug of choice for treating cryptococcal meningitis (Sar *et al*., 2004). All the three *Cryptococcus neoformans* isolates obtained from CSF were susceptible to all the antifungal agents tested. This is similar to the results obtained from Cambodia (Sar *et al*., 2004) where all the *Cryptococcus neoformans* strains they isolated from CSF were susceptible to amphotericin B. but in the USA ≤ 1% resistance to amphotericin B, fluconazole and flucytosine was reported (Pfaller *et al*., 2005).

Voriconazole showed an excellent *in vitro* activity against all the yeasts where no resistant strains were detected against it, but two isolates of *Candida albicans* were found to be in the intermediate category or dose dependent. These findings are similar to results obtained in Italy (Mandras *et al*., 2009) and in United States (Baran *et al*., 2000) where both studies found voriconazole to be highly active and found no resistant strains of *Candida* against it. However in Turkey, about 5.45% *Candida* isolates studied were reported to be resistant to voriconazole (Tulumoglu *et al*., 2009). Similarly resistance to fluconazole and other azoles (posaconazole, and voriconazole) and echinocandins (anidulafungin, caspofungin, and micafungin) was found to be low (<5%) in community bloodsream isolates in the USA, but resistant strains were most prevalent among nosocomial bloodstream infections of *C. glabrata* (Pfaller *et al*., 2011c).

The antifungal susceptibility levels reported are low in many countries (Pfaller *et al*., 2011a; Pfaller *et al*., 2011b), but the differences in susceptibility levels in these countries support the idea that the susceptibility of yeasts to antifungal drugs needs to be monitored and especially in Ghana, where resistance levels (≤22.4%) detected is above what is reported (Pfaller *et al*., 2005) worldwide.

5. CONCLUSION

In this study *Candida albicans* was the most common yeast isolated from all the clinical samples tested except CSF in which *Cryptococcus neoformans* was the commonest. The overall resistance level of the isolates to flucytosine, amphotericin B, fluconazole and itraconazole ranged from 4.5% to 22.2% giving a cause for concern. Voriconazole was the best performing antifungal drug to which no resistant yeast isolate was found. This high level of resistance in Ghana calls for further investigation taking into account the new clinical

breakpoints suggested. This is the first report on the yeast types and their antifungal susceptibility patterns in Ghana.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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