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An investigation of the distribution of *Candida* species in genitourinary candidiasis and pelvic inflammatory disease from three locations in Ghana

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The aim of this study was to determine the frequency of various *Candida* species in cases of genitourinary candidiasis and pelvic inflammatory disease (PID) in Ghana. To achieve this, *Candida* isolates were recovered from high vaginal swabs of patients with vulvovaginal candidiasis (VVC), urine samples from patients with urinary tract infections (UTIs) and endocervical swabs from patients with PID, from three teaching hospitals in Ghana. The hospitals were located at Korle Bu, Komfo Anokye and Tamale. These isolates were identified to the species level on the basis of the color of the colonies that they formed on chromogenic medium. *Candida albicans* was the most common species in high vaginal swabs from patients present for the first time with VVC in each of the three locations, and was present in 53.4% of the total swabs. The other species that were present were *Candida glabrata* (21.6%), *Candida parapsilosis* (15.5%), *Candida tropicalis* (4.7%) and *Candida krusei* (4.7%). Only single species were found in these swabs. In patients with VVC for at least the third time, Chi square analysis indicated that the frequency of each of these species were not statistically different from those present for the first time, although 15% of the swabs from these patients contained more than one species. For all patients with VVC, no significant differences were observed in the frequencies of the species between the three locations. Similar distributions were found in swabs taken from patients with PID. Across the three locations however, there was a significant difference in the frequency of *C. albicans*, which was present in 68 and 69.6% of patients from Komfo Anokye and Tamale, but only 26.7% of patients from Korle Bu. Twenty one percent of swabs from patients with PID contained more than one species. Urine samples were also collected from two of the locations, Korle Bu and Tamale, in female patients with candiduria. In Korle Bu, *C. glabrata* was the most prominent species (37.8%) followed by *C. albicans* (22.4%), *C. parapsilosis* (21.7%), *C. tropicalis* (10.5%), *C. krusei* (7%) and *Candida lusitaniae* (0.7%). In Tamale, the species distribution was *C. albicans* (60.9%), *C. glabrata* (21.7%), *C. parapsilosis* (13%) and *C. krusei* (4.3%). Statistical analysis indicated a significant difference in the frequency of *C. albicans* between the two locations. Fourteen percent of the urine samples contained more than one species. Taken as a whole, these data highlight a relatively high prevalence of species other than *C. albicans*, in cases of genitourinary candidiasis and PID in Ghana. This is consistent with a trend towards the emergence of other *Candida* species that may be more resistant to the first line antifungal treatments.

Key words: Genitourinary candidiasis, *Candida albicans, Candida glabrata, Candida parapsilosis*, Vulvovaginal candidiasis, Ghana.

INTRODUCTION

Candida infections of the genitourinary tract are of interest to clinicians as they can occur in both Immuno-compromisedand immunocompetent individuals. Such infections can have significant effects on morbidity; symptoms of vulvovaginal candidiasis (VVC) for example include local irritation, burning and pruritus, all of which can contribute

to significant discomfort and emotional distress. Furthermore, they have a high prevalence; Sobel (1998) has estimated that world-wide approximately 75% of women of child bearing age will suffer at least one occurrence of VVC and that half of these will have multiple occurrences.

While *C. albicans* is the most frequently isolated species in VVC, it is becoming evident that other species of *Candida* may be important (Enwuru et al., 2008). This is troubling, given that these other species may have higher resistance levels to azole-based treatments, and as such may be more prevalent in those experiencing recurrent episodes of VVC (Achkar and Fries, 2010). The emergence of non-albicans species appears to be espe-cially marked in African and Asian countries with *C. glabrata* accounting for up to 37% of cases in India (Ahmad and Khan, 2009). Similarly, *C. tropicalis* has been shown to be responsible for 18% of cases of VVC in Nigeria (Okungbowa et al., 2003) and *C. parapsilosis* for 10% of cases in India (Ahmad and Khan, 2009). These contrast with reported values of between 3 and 16% for *C. glabrata*, 1-2% for *C. tropicalis* and 1-4% for *C. parapsilosis* in other parts of the world (Achkar and Fries, 2010). It is thought that the prevalance of these non *C. albicans* species may be increasing over time (Chaim et al., 1997; Spinillo et al., 1997).

The incidence of species other than *C. albicans* in cases of candiduria is even higher and this appears to be irrespective of geographical location. Specific examples include *C. glabrata* which has been shown to be responsible for 53% of cases of candiduria in renal transplant patients in Wisconsin (Safdar et al., 2005) and *C. tropicalis* which was responsible for 36% of cases of candiduria in intensive care units in Spain (Alvarez-Lerma et al., 2003). Candiduria is typically asymptomatic and may be thought of as a somewhat benign infection; nevertheless it can significantly increase the costs of hospitalization, relative to other urinary tract infections (Achkar and Fries, 2010). There have been few studies of *Candida* species in Ghana or indeed in North West Africa as a whole. This is problematic, given that a rich variety of species including *C. albicans, C. glabrata, C. tropicalis, Candida dubliniensis, C. krusei, Candida sake, Candida guilliermondii* and *C. parapsilosis* have recently been described in samples taken from patients in a teaching hospital in Ghana (Feglo and Narkwa, 2012). The aim of this study was to expand on these findings and to identify and characterize *Candida* species in patients with infections of the lower and upper reproductive tract with VVC or PID and urinary tract infections from three major teaching hospitals in different parts of Ghana. Those with VVC were separated into patients that were present for the first time and patients

MATERIALS AND METHODS

Samples were collected from mid-January to April 2012 from the teaching hospitals at Korle Bu, Komfo Anokye and Tamale. These are located in the Coastal, Southern and Northern parts of Ghana, respectively. For all patients basic demographic data such as age and gender as well as information on the clinical condition were recorded from the patient's laboratory requisition form.

The high vaginal or endo-cervical swabs were taken by a trained clinical technician using sterile cotton tipped wooden swab sticks (Evepon industries limited, NAFDAC) from patients with either first time or recurrent vaginal discharge and from patients with suspected pelvic inflammatory disease. Two samples were taken with cotton tipped swabs from these patients. A few drops of normal saline were added to one of the swabs in a sterile tube which was then vortexed to form a suspension. A drop of the suspension was transferred onto a sterile glass slide for direct wet mount examination for the presence of yeast-like cells.

Urine specimens were processed as described by Feglo and Narkwa (2012). Mid stream urine specimens were taken from patients with suspected urinary tract infections after explanation of the urine collection procedure to them. Upon receipt of the urine specimens, they were transferred into a sterile centrifuge tube and any yeast cells were collected by centrifugation. A loopful of sedimented cells was transferred onto a glass slide for direct wet mount preparation for the presence of yeast-like cells.

Preliminary microscopic screening was performed on all the specimens using a direct wet mount preparation made up of 10% potassium hydroxide (KOH) and a drop of 1% w/v lactophenol cotton blue. The presence of typical colonial oval or ellipsoidal yeast-like morphology was used as an indicator for the presence of yeast. All yeast-like positive isolates recovered from clinical specimens were cultured on Sabouraud dextrose agar (SDA).

For culturing, a loopful of the urine sediment (using a 0.01 ml of an inoculating wire loop) or the second swabs from each yeast-like positive specimen were inoculated onto an SDA plate supplemented with 50 mg/L gentamicin and chloramphenicol using the streak-out plate method. Inoculated SDA plates were incubated aerobically at 37°C for 48 h. The plates were examined for the presence of growth after 48 h of incubation. Colonies that were raised, had an opaque and creamy colour and those with distinct yeast-like odour were preliminarily tagged as *Candida* species. Swabs that gave cultured specimen plates that were three quarters full of *Candida* colonies and urine specimens with *Candida* colonies more than 100 CFU/ml were used for this study. Colonies were purified by a repeated streaking method on the same medium to obtain pure discrete colonies. These were subsequently grown and maintained on an SDA slant at 4°C and were transported to the School of Biological Sciences, University of Canterbury, Christ church, New Zealand for species identification.

For identification, the clinical specimens were inoculated onto chromogenic media (Brilliance Candida agar; BCA) plates, incubated aerobically at 37°C and examined for the presence of pigmented colonies after 72 h as described by Jabra-Rizk et al. (2001). For uniformity and reproducibility of results, cell density was adjusted to 10^6 cells/ml using the relevant dilution. A 10 µl drop of a cell suspension was inoculated onto one side of the BCA plate and was spread evenly over the entire surface of the plate with a sterile inoculating loop. After incubation, isolates were characterized into their respective species type based on manufacturer's colour code. The following colours and colony morphologies were used to describe each *Candida* species present: *C. glabrata* (light pink colonies, wet), *C.tropicalis* (blue colonies, wet), *C. krusei* (rough, spreading colonies with pale pink centres and white edges dry), *C. albicans* (green colonies, wet) and *C. parapsilosis* (golden brown, wet). Further confirmatory tests to differentiate between *Candida albicans* and non-albicans species were chlamydospore formation on corn meal- Tween 80 agar as described by McGinnis (1980), the formation of a germ tube in serum as described by Coleman et al., (1997), Odds and Bernaerts (1994), Pincus et al. (1999) and whether

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Table 1. Distribution of *Candida* species in high vaginal swabs from patients present for the first time with a vaginal discharge in three locations in Ghana. Data are presented as the number of swabs in which each species was present and the percentage that number represents.

Species	Number and percentage of HVS containing each species			Total (n=148)
	Korle Bu (n=58)	Komfo Anokye (n = 59)	Tamale (n=31)	
C. albicans	26 (44.8%)	34 (57.6%)	19 (61.3%)	79 (53.4%)
C. glabrata	16 (27.6%)	9 (15.3%)	7 (22.6%)	32 (21.6 %)
C. parapsilosis	8 (13.8%)	10 (16.9%)	5 (16.1%)	23 (15.5 %)
C. tropicalis	4 (6.9%)	3 (5.1%)	0	7 (4.7 %)
C. krusei	4 (6.9%)	3 (5.1%)	0	7 (4.7%)

Table 2. Distribution of *Candida* species in high vaginal swabs from patients that have previously presented on at least three previous occasions with vaginal discharge in three locations in Ghana. Data are presented as the number of swabs in which each species was present and the percentage that number represents.

Species	Number and percentage of HVS containing each species			Total (n=86)
	Korle Bu (n=43)	Komfo Anokye (n=23)	Tamale (n=20)	
C. albicans	15 (34.9%)	13 (56.5%)	13 (65%)	41 (47.7%)
C. glabrata	11 (25.6%)	4 (17.4%)	6 (30%)	21 (24.4%)
C. parapsilosis	12 (27.9%)	4 (17.4%)	0	16 (18.6%)
C. tropicalis	4 (9.3%)	1 (4.3%)	1 (5%)	6 (7%)
C. krusei	1 (2.3%)	1 (4.3%)	0	2 (2.3%)

or not growth occurred at 45°C as described by Pinjon et al. (1998).

All media used were from Oxoid, Limited, Basingstoke, UK unless otherwise specified. Species from the American type culture collection (ATCC), *C. albicans* 10231, *C. parapsilosis* 90018, *C. tropicalis* 13803, *C. krusei* 6258, *C. glabrata* 2001, *C. kefyr* 4135 and *C. lusitinae* 34449 were used as controls. These were kindly given by the Canterbury District Health Board.

Statistical analysis was conducted using Pearson's Chi-squared test in SPSS version 17 on the observed and expected frequency of the various *Candida* species. A significant level for Chi (X^2) is given by a probability ($P < 0.05$).

RESULTS

There are various methodologies that can be used for identification of *Candida* species. In our studies, *Candida* species were identified primarily on the basis of chromogenic tests although, as detailed above additional testing was done to confirm the chromogenic data differentiating between *C. albicans* and the other species. As expected only those isolates identified as *C. albicans* on the basis of the chromogenic tests were found to grow at 45°C, to form chlamydiospores on CMA-Tween 80 media, and to form germ tubes in human serum. Thus, differentiation of *C. albicans* from non-albicans species was consistent irrespective of the methodology used. The chromogenic tests identified 6 *Candida* species in total, *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis*, *C. krusei* and *C. lusitinae*.

The species distribution in high vaginal swabs from patients with VVC for the first time is shown in Table 1. *C. albicans* was most common in each of the three locations, present in over half of the total swabs (53.4%). The next most common species were *C. glabrata* (21.6%), *C. parapsilosis* (15.5%), *C. tropicalis* (4.7%) and *C. krusei* (4.7%). Only single species were isolated from these patients. A Chi square test showed no significant difference ($X^2 = 7.76$ $P=0.46$, d.f. = 8) between the species frequencies in the three locations. Similarly there was no significant difference ($X^2 = 11.16$, $P=0.19$, d.f, = 8) between the species frequencies in the three locations for patients who were with VVC for at least the third time (Table 2), nor when comparison were made between the frequency of each species in patients present for the first time and for three or more times with VVC ($X^2 = 7.71$, $P=0.46$, d.f. = 8). In 15% of the patients with VVC three or more times, more than one species was present, the most common combinations being patients with combinations of *C. albicans* and *C. parapsilosis* (4%), *C. albicans* and *C. glabrata* (3%), and *C. glabrata* and *C. parapsilosis* (3%). In Korle Bu 24% of patients had more than one species, this compares with figures of 13 and 4%, respectively, for Komfo Anokye and Tamale. A sample from one patient in Korle Bu contained three species (*C. albicans*, *C. glabrata* and *C. parapsilosis*).

Swabs taken from patients with PID in the three locations showed a significant difference ($X^2 = 19.95$, $P < 0.01$, d.f. = 8) with respect to the frequency of *C. albicans* which was present at a lower frequency in Korle Bu as compared to Komfo Anokye and Tamale (Table 3). Twenty one percent of patients with PID had more than one species present, the most common combinations being *C. albicans* and *C. parapsilosis*, *C. albicans* and *C.*

Table 3. Distribution of *Candida* species in endocervical swabs from patients with pelvic inflammatory disease in three locations in Ghana. Data are presented as the number of swabs in which each species was present and the percentage that number represents.

Species	Number and percentage of endo-cervical swabs containing each species			Total (n=93)
	Korle Bu (n=45)	Komfo Anokye (n=25)	Tamale (n=23)	
C. albicans	12 (26.7%)	17 (68%)	16 (69.6%)	45 (48.4%)
C. glabrata	15 (33.3%)	4 (16%)	6 (26.1%)	25 (26.9%)
C. parapsilosis	11 (24.4%)	2 (8%)	1 (4.3%)	14 (15.1%)
C. tropicalis	4 (8.9%)	1 (4%)	0	5 (5.4%)
C. krusei	3 (6.7%)	1 (4%)	0	4 (4.3%)

Table 4. Distribution of *Candida* species in "mid stream" urine of female patients with a urinal tract infection in Korle Bu and Tamale. Data are presented as the number of urine samples in which each species was present and the percentage that number represents.

Species	Number and percentage of urine samples containing each species		
	Korle Bu (n=143)	Tamale (n=23)	Total (n=166)
C. albicans	32 (22.4%)	14 (60.9%)	46 (27.7%)
C. glabrata	54 (37.8%)	5 (21.7%)	59 (35.5%)
C. parapsilosis	31 (21.7%)	3 (13%)	34(20.5%)
C. tropicalis	15 (10.5%)	0	15 (9%)
C. krusei	10 (7%)	1 (4.3%)	11 (6.6%)
C. lusitaniae	1 (0.7%)	0	1 (0.6%)

glabrata, and *C. parapsilosis* and *C. tropicalis* (each combination present in 3% of patients).

In addition to the swabs taken from the reproductive tracts, we tested urine from female patients that presented with urinary tract infections. We were only able to collect and test urine samples from two of the locations, Korle Bu and Tamale (Table 4). As was the case for patients with PID, there was a significant difference (X^2 = 15.54, P<0.01, d.f. = 8) in the frequency of *C. albicans* in samples from the two locations (Table 4) and indeed in Korle Bu, *C. glabrata* was actually the most prominent species (37.8%) followed by *C. albicans* (22.4%). Fourteen percent of the urine samples contained more than one species.

DISCUSSION

The data indicate that *C. albicans* is, in most instances, the most prevalent species in the cases of genitourinary candidiasis in the three locations that samples were taken from. However, it should be noted that other *Candida* species were responsible for a significant number of cases, in particular *C. glabrata* and *C. parapsilosis*. This is consistent with the findings of other studies in North West Africa, India and Turkey.

C. glabrata has been shown to account for 37% of cases of VVC in India (Ahmad and Khan, 2009) 34% of cases in Nigeria (Okungbowa et al., 2003), 30% of cases in Turkey (Cetin et al., 2007) and 18% of cases in a previous study at Komfo Anokye (Feglo and Narkwa, 2012). We report values of between 15.3 and 27.6% with an overall presence in 21.6% of patients present for the first time, and values of between 17.4 and 30% with an overall frequency of 24.4% for patients who have presented on at least three previous occasions. These contrast with values of between 3 and 16% in the US, Australia and China (Achkar and Fries, 2010). Similarly, *C. tropicalis* has previously been shown to account for 18% of cases of VVC in Nigeria (Okungbowa et al., 2003) and *C. parapsilosis* for 10% of cases in India (Ahmad and Khan, 2009). These values are comparable to those of the present study, and that of Feglo and Narkwa (2012), and again these are higher than the reports of 1-2% for *C. tropicalis* and 1-4% for *C. parapsilosis* elsewhere in the world (Achkar and Fries, 2010).

There was no significant difference in the species frequencies in patients with VVC for the first time, when compared with those who had presented previously. We are unaware of any previous studies that make this comparison. Given that the emergence of non-albicans species has been attributed to their greater tolerance of azole-based treatments, it might have been expected that there would be an increase in these species in the patients that had presented previously. Unfortunately we are unsure what, if any, treatments were given with their

earlier presentations, as we were not able to attain their previous clinical data. Even with that data however, there is no guarantee that the patient took/completed their course of treatment. Clearly, this is an area where further research is warranted and it is note-worthy that there were patients with multiple species present in patients that had presented previously while in those present for the first time there were only single species present. This suggests that previous infections/treatment regimes may make patients more susceptible to a mixed flora.

In addition to infections of the lower reproductive tract, micro organisms may also infect areas above the cervix, giving rise to a variety of conditions that are described under the generic term pelvic inflammatory disease (PID). Such infections affect about 1 million women in the US annually, about a quarter of whom may suffer some form of serious long term complication such as infertility (Pletcher and Slap, 1998). While bacteria such as *Chlamydia trachomatis* and *Neisseria gonorrhoea* appear to be the primary species responsible for these infections there have been reports of *C. albicans* in 23% of endocervical swabs in a study in Nigeria (Audu and Kudi, 2004). Our data suggest that other *Candida* species may also be present and indeed the frequency of *C. albicans* may differ depending on the location. It should be noted however, given that the *Candida* species are much more commonly associated with VVC rather than PID, that there is the potential for contamination of the endo-cervical swabs by organisms in the vagina.

The prevalence of non-*Candida albicans* species in cases of Candiduria is consistent with reports in Africa and other places around the world with *C. glabrata,* for example, accounting for 53% of cases in renal transplant patients in parts of the US (Safdar et al., 2005). This is similar to the situation in Korle Bu where *C. glabrata* was present in more urine samples than *C. albicans.* In contrast, *C. albicans* was the most prevalent species in urine samples taken from patients in Tamale. Odds (1988) has suggested that differences in any underlying diseases, antimicrobial and chemotherapeutic practices in patients might have led to variation in the type of *Candida* species isolated from institution to institution. There may also be climatic and cultural differences between Korle Bu and Tamale contributing to the different species distributions. Korle Bu is located in Accra, the capital city of Ghana, an industrialized and cosmopolitan city with a largely Christian population. Tamale is located in northern Ghana about 430 km north of the capital, it has a much hotter and drier climate and prone to Harmattan winds. It is inhabited by predo-minantly Islamic communities.

In this study, no *C. dubliniensis* isolates were recovered among our clinical samples. This species had previously been reported in South Africa, Egypt and Tunisia as well as Ghana (Al Mosaid et al., 2003; Bii et al., 2009; Jabra-Rizk et al., 2001; Kwamin et al., 2010). This may be due to, in part, the type of samples used in the study; urine and vaginal specimens have been reported to have a low incidence of *C. dubliniensis* (Loreto et al., 2006).

In summary we present data that indicates a relatively high prevalence of species other than *C. albicans* in cases of genitourinary candidiasis and PID from three locations in Ghana. This is consistent with a reported trend towards the emergence of other *Candida* species, especially *C. glabrata* and *C. parapsilosis*. This has implications for diagnostic laboratories which are reliant on tests that are specific for *C. albicans*.

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