IDENTIFICATION AND CHARACTERIZATION OF CANDIDA SPECIES ISOLATED FROM CLINICAL SPECIMENS IN ILORIN, KWARA STATE

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Abstract

Candida has been reported has one of the most prevailing yeast genera. Candida is of diverse species that are commonly leave on soil and in the mucosal surfaces of genitourinary tract, gastrointestinal tract, and the mouth of human and have ability to cause oral thrush or vaginal thrush. The incidence of Candida infections has dramatically increased in recent years due to a significant increase in cases like HIV/AIDS, cancer, diabetes mellitus, long time use of antibiotic and tissue transplant. It is a cross-sectional study involving patients diagnosed of Candidiasis. The aim of this study is to identify, characterize and determine the prevalence of Candida species isolated from clinical specimens in selected health care facilities in Ilorin, Kwara state, Nigeria using conventional methods such as CHROM agar Candida differential media and Germ tube test. Of the 232 isolates recovered from Sabouraud dextrose agar, sub-cultured on CHROM agar and tested for Germ Tube Test. C. albicans 131(56.5%), has the highest prevalence, non-albicans such as C. tropicalis has the prevalence of 36(15.5%), C. dubliniensis 27(11.6%), C. glabrata 14(6.0%), C. krusei 12(5.2%), there was mixed growth 7(3.0%) and 3(1.3%) did not grow on CHROM agar. Candida albicans is the most prevalence of Candida species isolate from clinical specimen in Ilorin, Nigeria in this study (56.5%) and C. albicans is also the most prevalence in Female (59.6%) while C. tropicalis has the highest prevalence in Male (36.8). Age group between 20 -29 has the highest prevalence (64.5%). Clinical specimens that produce highest number of Candida species in this study was High Vaginal Swab (58.2%) and pelvic inflammatory disease was the most clinical diagnosis (34.1%) and female subject has the highest prevalence of Candida infections (91.8%) while male has (8.2%) in Ilorin, Nigeria. There was a statistical significant difference (P < 0.01) between age and Candida species distribution among participant.

KEY WORDS: Candid species, Candidiasis, Fungi infection, Clinical infections, CHROM agar

1. Introduction

Candida has been reported has one of the most prevailing yeast genera. [15]. Candida is of diverse species that are commonly live on soil and in the mucosal surfaces of genitourinary tract, gastrointestinal tract, and the mouth of human and have ability to cause oral thrush or vaginal thrush. [11]. At least 15 species of Candida cause diseases in human [16], however, Candida albicans has been reported as the most frequent species causing infection of human [8]. C. glabrata, C. krusei, C. parapsilosis C. tropicalis among others have also been reported as species that cause human infections [14].

Most of these Candida species are used to be normal inhabitant of the mucous membranes and the skin, however. under favuorable conditions. these organisms develop virulence factors which make them infectious or opportunistic in patients with debilitating clinical condition [3], or due to the use of invasive devices [5], or Immunocompromised individual. [18]. Due to the increased in the prevalence of topical and invasive diseases caused by Candida new clinical syndromes have emerged, the expression of which depends solely on the immune status of the host [11]. Candida species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to systemic illnesses, the clinical manifestations may be acute, sub-acute or

chronic to episodic, involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis and meningitis [11]. C. albicans is the most common vaginal isolate with a prevalence of 70%-90% while non-albicans Candida species such as, C. glabrata (Torulopsis glabrata), C. tropicalis, C. dubliniensis, C. krusei, C. famata, C. parapsilosis, kefyr (C. pseudotropicalis), and *C. lusitaniae* are less frequent [10]. However, a significant increase in recurrent Candida vaginitis has been reported to cause by significant increase in non-albicans Candida species associated with formation of virulence factors. [2]. In recent years, the number of yeast infections seems to have increased significantly worldwide [1]. Furthermore, due to a large increase in HIV/AIDS cases, the frequency of Candida infections has dramatically elevated, thus an ever-expanding population with immuno-compromise due to mucosal or cutaneous barrier disruption, defects in the number and function of neutrophils or in cell-mediated immunity, metabolic dysfunction, organ transplantation and extremes of age <1 year and >70years [13]. Although Candida albicans still remains responsible for most yeast infections, non-albicans species appear to be increasing in prevalence [6]. The greatest relevance of these recent changes in the distribution and epidemiology of severe Candida infections lies in the intrinsic differences between each of these species in their susceptibility to antifungal therapies [7]. Candida infections in age groups between 23-25 are reported to have prevalence of 25.0% in Ilorin. [12]. The Speciation of Candida helps in identification of those species which might be intrinsically resistant to some of the antimycotics [9]. Ordinarily, the speciation of Candida isolates is done by wet mount, Gram staining technique, germ tube test, sugar fermentation and sugar assimilation test [4]. API systems, CHROM agar, molecular methods and Vitek 2 ID system have been the newer methods [9]. Among the newer methods, CHROM agar is rapid and cost effective as compared to other expensive systems like API systems, Vitek 2 ID system and molecular methods [17].

However, *Candida* species characterization is important for the management of candidiasis [11]. Speciation aids in understanding of epidemiology of infection specifically the source and mode of transmission of the infection which enhances the development of adequate measures to prevent and control transmission of resistant strains [11].

Therefore, this study shall identify and characterize the most common *Candida* species and the distribution of *Candida* species in clinical specimens in selected health care facilities in Ilorin, Kwara state, Nigeria.

2. Materials and Methods

Study Area: The study area includes both inpatients and outpatients which *Candida* species was isolated from their clinical specimen across selected health care facilities in Ilorin, Kwara state.

Study design: It is a cross-sectional study involving patients diagnosed of Candidiasis.

Ethical approval: Ethical approval was obtained from the Kwara State ministry of health, Ilorin (MOH/KS/EU/777/559) prior to the commencement of this study.

Sample size: The sample size was determined by the formula described by (Fisher *et al.*, 1998).

 $N = \underline{Z^2 P Q}$

- D^2 N = sample size
- Z = 1.96 (for 95% confidence level)
- P = Prevalence
- O = 1 P
- D = 0.05 (confidence level or tolerance error)

Prevalence of Candidiasis in Ghana is 21.0% (Abruquah, 2012).

Z = 1.96, c=0.05, p = 21.0= (0.21).

 $N = \frac{1.96^2 \times 0.21(1 - 0.21)}{2.54} = 2.54$

Study population: Candida growths from clinical specimens of inpatients and outpatients in selected health care facilities.

Duration of study: The study lasted for a period of Three (3) months from sample collection to the analysis of the samples.

Inclusion Criteria: Clinical specimens that yielded *Candida* growth.

In and out patients in health care facilities in Ilorin, kwara State.

Exclusion Criteria: Specimens Collected in health care facility out of Ilorin Kwara state.

Sampling Techniques: Clinical specimens such as High Vaginal Swab, Endocervical Swab, blood culture, sputum urine or nail scrapping collected from the patients were cultured and those that yielded *Candida* growth were isolated.

N = 254. 0.05²

Data Collection Procedure: Demographic, socioeconomic and medical histories were obtained from patients using the health care facilities' patients' laboratory register. All information collected was not linked to patients' personal, health or treatment records. Code was used to reference participant sample so that participants will not be traceable via collected data or specimens.

Laboratory procedure: Gram staining technique was used to determine the gram reaction. Germ tube test was also done to differentiate *C. albicans* from *C. dubliniensis* and the isolates were sub-cultured on CHROM agar for further speciation.

Data analysis: The data generated from this study was analyzed by the statistical software SPSS version 24 (SPSS) for windows. The statistical testing was used to evaluate the epidemiological survey of *Candida species* isolated from clinical specimens in Kwara state. P- Value < 0.01 was taken to be statistically significant.

3. Result

Clinical diagnoses of *Candida* infections among the participants

Table 1 shows the clinical diagnoses of *Candida* infections among the participants with Pelvic inflammatory disease having the highest frequency 79 (34.1%), followed by Vulvovaginal candidiasis 67 (28.9%), HIV/AIDS 28 (12.1%), Sepsis 14(6.0%), Premalignant lesion 11 (4.7%), Urinary tract infection 10 (4.3%), Respiratory tract infection 7 (3.0%), infertility 6 (2.6%), urosepsis 4 (1.7%), each of Acute kidney infection and Dermatophytosis has 2 (0.9%), while each of septic shock and Degenerative disease of spine has 1 (0.4%).

Distribution of *Candida* species in Clinical **Specimens** Table 2 shows the distribution of *Candida* species in clinical specimens with highest number of *Candida* species 135 (58.2%) isolated from High Vaginal Swab (HVS), 28 (12.1%) were isolated from Endocervical Swab (ECS), 27(11.6%) were isolated from oral swab, 18(7.8%) were isolated from urine, 12 (5.2%) were isolated from blood, (3.0%) were isolated from sputum, 2(0.9%) were isolated from each of wound swab and nail scrapping while 1(0.4%) was isolated from throat swab.

Gender distributions of *Candida* species

Table 3 shows the gender distribution of *Candida* species. *C. albicans* appears most in female 127(59.6%), followed by *C. tropicalis* 28(13.1%), *C. dublinesis* 26 (12.2%), *C. glabrata* 13(6.1%), *C.*

krusei 10(4.7%) while *C. paraplosis* appears least 1(0.5%). However, in male *C. tropicalis* appears most 7(37.8%), followed by *C. albicans* 6(31.6%), *C. dubliniensis* 2(10.5%), *C. glabrata* and *C. Krusei* each appears 1(5.3%) while *C. paraplosis* also appears least in male 1(0.5%). There was statistically significant difference (p- value <0.01) between the gender and *Candida* species distribution.

Age distribution of *Candida* species

Table 4 shows the age distribution of Candida species. In age group between 0-9 years, C. albicans appears 1(25.0), C. tropicalis appears 2(50.0%) C. krusei appears 1(25.0%) while each of C. glabrata and dubliniensis appears 0(0.0%). In age group between 10-19 years, C. albicans appears 2(40%), mixed growth of C. krusei/glabrata 2(40%), C. dubliniensis 1(20%). In age group between 20-29 years, C. albicans appears most with prevalence of 66(63.3%), followed by C. tropicalis 9(8.9%), C. glabrata 2(2.0%), each of C. dubliniensis and C. krusei 4(4.0%), and C. paraplosis 0(0.0%). In the age group between 30- 39, C. albicans appears most with the prevalence of 40(54.8%), followed by each of C. dubliniensis and C. krusei 3(4.1%), C. paraplosis 2(2.7%) and C. glabrata 0(0.0%), For the age group between 40 and above, C. albicans has the highest prevalence of 2(46.2), C. tropicalis 15(28.8%), C. krusei has the prevalence of 5(9.6%), C. dubliniensis 5(9.6%) while each of C. glabrata and C. paraplosis has 0(0.0%). There was a statistical significant difference (P < 0.01) between age and Candida species distribution among participants.

Prevalence of *Candida* species isolated from clinical specimen

Table 5 shows the prevalence of *Candida* species isolated from clinical specimen. Of the 232 isolates recovered from Sabouraud Dextrose Agar (SDA), sub-cultured on CHROM agar and tested for Germ Tube Test, *C. albicans* has the highest prevalence of 131(56.5%), non-albicans such as *C. tropicalis* has the prevalence of 36(15.5%), *C. dubliniensis* 27(11.6%), *C. glabrata* 14(6.0%), *C. krusei* 12(5.2%), there was mixed growth 7(3.0%) and 3(1.3%) did not grow on CHROM agar.

Diagnosis	Frequency	Percentage
	(n)	(%)
Pelvic Inflammatory	79	34.1
Vulvovaginal	67	28.9
HIV/AIDS	28	12.1
Infertility	6	2.6
Sepsis	14	6.0
Urinary Tract	10	4.3
Acute Kidney	2	0.9
Respiratory tract	7	3.0
Septic Shock	1	0.4
Degenerative disease	1	0.4
Dermatophytosis	2	0.9
Premalignant lesion	11	4.7
Urosepsis	4	1.7
Total	232	100.0

Table 1; Clinical diagnoses Distribution of Candida infections among the participants

	Frequency	Percent
Clinical Specimen		
High Vagina Swab	135	58.2
Endo-Cervical Swab	28	12.1
Oral Swab	27	11.6
Blood	12	5.2
Urine	18	7.8
Sputum	7	3.0
Wound Swab	2	0.9
Throat Swab	1	0.4
Total	232	100.0

Table 2; Distribution of Candida species in Clinical Specimens

KEY: Correlation is

the 0.01 level (2-tailed) < 0.01

significant at

Candida species	Female	Male	Total	p-value
C. albicans	127(59.6)	6(31.6)	133(53.3)	
C. glabrata	13(6.1)	1(5.3)	14(6.0)	
C. dubliniensis	26(12.2)	2(10.5)	28(12.1)	
C. krusei/ albicans	2(0.9)	0(0.0)	2(0.9)	
C.krusei	10(4.7)	1(5.3)	11(4.7)	0.208**
C. tropicalis	28(13.1)	7(37.8)	35(15.1)	
No Growth	3(1.4)	0(0.0)	3(1.3)	
C. paraplosis	1(0.5)	1(5.3)	2(0.9)	
C. tropicalis/albicans	1(0.5)	1(5.3)	2(0.9)	
C. albicans/paraplosis	1(0.5)	0(0.0)	1(0.4)	
C. krusei/glabrata	1(0.5)	0(0.0)	1(0.4)	
Total	213(100)	19(100)	232(100)	

Table 3; Gender distributions of Candida species

 Table 4; Age distribution of Candida species

C 1:1 :	0.0	10.10	20.20	20.20	<u> </u>
Canalaa species	0-9	10-19	20-29	30-39	Above 40
C. albicans	1(25)	2(40.0)	66(63.3)	40(54.8)	24(46.2)
C. tropicalis	2(50.0)	0(0.0)	9(8.9)	11(15.1)	15(28.8)
C. krusei	1(25)	0(0.0)	4(4.0)	3(4.1)	5(9.6)
C. glabrata	0(0.0)	0(0.0)	2(2.0)	0(0.0)	0(0.0)
C. dublinesis	0(0.0)	1(20.0)	4(4.0)	3(4.1)	5(9.6)
C. paraplosis	0(0.0)	0(0.0)	0(0.0)	2(2.7)	0(0.0)
C. tropicalis/albicans	0(0.0)	0(0.0)	0(0.0)	1(1.4)	1(1.9)
C. albicans/paraplosis	0(0.0)	0(0.0)	0(0.0)	1(1.4)	0(0.0)
C. krusei/albicans	0(0.0)	0(0.0)	0(0.0)	1(1.4)	0(0.0)
C.krusei/glabrata	0(0.0)	2(40.0)	5(5.0)	2(2.7)	5(9.6)
No Growth	0(0.0)	0(0.0)	2(2.0)	1(1.4)	0(0.0)
Total	4(100)	5(100)	101(100)	73(100)	52(100)

KEY: HS Highly Significant

Correlation is significant at the 0.01 level (2-tailed) <0.01 remark HS

Correlation is significant at the 0.05 level (2-tail)

Candida Species	Frequency (n)	Percent
Candida. albicans	131	56.5
Candida tropicalis	36	15.5
Candida dubliniensis	27	11.6
Candida glabrata	14	6.0
Candida krusei	12	5.2
Candida paraplosis	2	0.9
Mixed growth	7	3.0
No Growth Total	3 232	1.3

Table: 5 Prevalence of Candida species isolated from clinical specimens

4 Discussion

This study was carried out on Candida species from different clinical specimens of all age groups and gender in Ilorin, Kwara State, Nigeria. In this study a total of 232 Candida species were isolated from various clinical specimens. Age group between 20-29 years has the highest prevalence 101(43.5%), followed by 30-39 years age group 73(31.5%) C. albicans is the most prevalence in age group between 20-29 and 30- 39 with prevalence of (63.3%) and (54.8%) respectively (Table 4.4), which supports the previous study by (Oyeyipo et al., 2015) who reported high prevalence (25.0%) of candidiasis in age group 23-25 years in Ilorin and (Omosigho et al., 2019) who reported high prevalence in age group between 20-29 years followed by 30- 39 years in Bida, North Central, Nigeria. These age groups comprise of young women, sexually active with low vaginal defense mechanisms against Candida species. Most of the women within 20-30 years of age were mostly multiparous and use contraceptive which also favors candidiasis. However, female participants in this study has the highest prevalence of candidiasis 213 (91.8%) with male participants having 19 (8.2%).. This agrees with previous studies carried out by Omosigho et al. (2019) and Sjatha et al. (2015) which showed high prevalence of Candidiasis in female. This may be due to anatomical structure of females that provides favorable environmental conditions for the growth of Candida species such as increased physiological changes in estrogen and rich glycogen content of the vaginal mucosa which provided adequate supply of utilizable sugar that

support their proliferation. Also, the practice of vaginal douching by females increased the risk of vaginal candidiasis and frequent visit and long staying in the hospital by females lead to nosocomial infection. C. albicans is the most predominant species in the overall female subjects (59.6%), followed by C. tropicalis (13.1%), C. dubliniensis (12.2%), C. glabrata (6.1%), C. krusei (4.7%) and C. parapsilosis (0.5%) as indicated by (table 4.3). This agrees with previous studies by Omosigho et al. (2019), (Oyeyipo et al. 2015), Abruquah, (2012) and Enwuru, et al. (2008) who also reported high prevalence of C. albicans in female subjects, but disagrees with Charkabati et al.(1996) who reported a higher prevalence of 75.0% non-albicans Candida. It is interesting to note that Candida albicans was the most common species isolated (56.5%) in this study (Table 4.5). Similar results of C. albicans predominance has also been reported in various studies (Enwuru, et al., 2008; Omosigho et al., 2019; Oyeyipo et al., 2015). The high prevalence of C. albicans in this study (56.5%) and the high prevalence in C. albicans in female subjects (59.6%) could be as a result of ability of C. albicans to attach easily to the vaginal epithelial cells through their surface mannoprotein. Mannoprotein in the surface of C. albicans as well as germ tube formation and mycelium formation facilitate vaginal mucosal invasion. In addition, this may be linked to its virulent factors which include dimorphism and phenotypic switching. C. albicans are able to produces protease and phosphatase which enhance its

attachment to human epithelium. Moreover, it can be

deduced that the high incidence rate of *C. albicans* may be due to increased physiological changes in estrogen and rich glycogen content of the vaginal mucosa there by providing adequate supply of utilizable sugar that support its proliferation, hence the reason *C. albicans* is considered a major component of normal vaginal flora. However, there is correlation between microbial number and the onset of pathogenicity.

Among the non-albicans species, *Candida tropicalis* (15.5%), is the most predominant species in our study, followed by *C. dubliniensis* (11.6%) *C. glabrata* (6.0%) *Candida krusei* (5%) *C. parapsilosis* (0.9%). This study agrees with some previous studies (Omosigho *et al.*, 2019 and Oyeyipo *et al.*, 2015) in Bida and Ilorin respectively and supports the high prevalence of *Candida albicans* and a gradual shift towards non-albicans *Candida* species. Non- albicans play an important role in Vulvovaginal candidiasis, *C. tropicalis* and *C. glabrata* are not producing mycelia but they produce proteolytic enzymes that help the fungi to adhere to the vaginal epithelial cells thus, increasing the incidence of non-albicans *Candida* which has been reported in various studies.

In male subjects, C. tropicalis occurred most (37.8%), followed by C. albicans (31.6%) as indicated by (Table 4.3). This agrees with previous study by (Omosigho et al., 2019) who also reported highest frequency of C. tropicalis (88.9%) in male. The dominancy of C. tropicalis among non-albicans and high prevalence in male subjects may be due to the high genetic similarities C. tropicalis shares with C. albicans and ability to produce virulence factors such as adhesin, morphogenesis and phenotypic switching and biofilm formation. C. albicans has more adherent than other non-albicans species, but C. tropicalis is considered the second most adherent species of the Candida genus. There are considerably less studies concerning morphogenesis in other non- albicans species. Several Candida species may develop pseudo-hyphae, but quite a few are able to form true hyphae, including C. albicans, C. dubliniensis, and C. tropicalis. Although, C. tropicalis do not show the same degree of filamentation as C. albicans; however, because of the fact they are frequently associated with infectious processes, they certainly have mechanisms of adaptation that may favor filamentation in specific environmental conditions. Interestingly, C. tropicalis is the strongest biofilm producers, in fact, another study reported that the high thickness of the Extracellular Polymeric Substance (EPS) matrix of C. tropicalis biofilm cells may impair oxygen and nutrients diffusion to cells, and may be responsible for the lower metabolic activity.

Pelvic Inflammatory Disease is the most common clinical diagnosis in our study (34%) followed by Vulvovaginal candidiasis (28%) as typified by table 4.3 and Candida was mainly isolated from high vaginal swab (58.2%), Endocervical swab (12.1%) and Oral swab (11 .6%). Urine with 7.8%, blood 5.2%, sputum 3.0%, wound swab 0.9%, nail scrapping 0.9% and throat swab 0.4% (Table 4.2). The high frequency of high vaginal swab (58%) was as a result of the high occurrence of Vulvovaginal candidiasis (28%) and the high prevalence of candidiasis in female subjects range between the age group of 20-29 and 30-39 year (43.5%) and (31.5%) respectively in this study confirms the high prevalence of candidiasis in female of reproductive age group in Ilorin, Kwara State, Nigeria and this study agrees with the previous study by (Omosigho et al., 2019), who reported high prevalence of candidiasis in female subject within the age group of 20- 30 years in Bida North Central, Nigeria. The prevalence rate of vulvovaginal candidiasis in our study (28%) correlates very closely with the previous study by Kalia et al. 2015 (31%,) and lower than that of EA Ugwa (84.5%) in North-West Nigeria. Low socioeconomic status, improper hygiene, less education, and African ethnicity maybe the probable factors for high prevalence in Vulvovaginal candidiasis in Ilorin. In surveys, the prevalence of Vulvovaginal candidiasis is highest among women in their reproductive age. The prevalence increases with age up to menopause, it's higher in Africa and it decreases across the postmenopausal women, unless they are taking estrogen therapy and uncommon in prepubertal girls. However, varying prevalence could be due to multiple factors, including sociodemographic characteristics, immune status of patients, treating patients with broad spectrum antibiotics and immune suppressive drugs, and hormonal influences, etc.

Also, Oral swab has the third highest frequency (11.6%) among clinical specimens in this study with clinical diagnosis of HIV/AIDs. 20 out of 27 (74%) of oral swab were diagnosed for HIV/AIDs and all yielded growth of Candida species. This shows the clinical correlations between Oral Candidiasis and HIV/AIDs. Early in the HIV epidemic, oral candidiasis was found to be among the strongest clinical predictors of progression to AIDS in various HIV-infected populations. Oral candidiasis has also been shown to be the most common oral manifestation of HIV infection in different parts of the world and to be closely associated with low CD4+ cell count. A lower count, especially below 200 CD4+ cells with or without statistical significance has been frequently associated with the increased occurrence of Oral candidiasis.

Our study also reported 3% mixed growth. The isolation of mixed growth in this study shows the possibility of isolating two or more species of Candida from a single clinical specimen (Table 4.5). Although, this has been previously isolated from oropharyngeal swab of HIV infected patients as reported by various studies. Ho et al. (2014) reported that among the 45% of HIV outpatients colonized by yeasts, 16.5% harbored more than one species. Interestingly, Menezes et al. (2015) reported that 77.5% of the cases had Candida colonization by a single species, whereas 22.5% of the cases had a combination of two or more species. Similarly, in our study some of the oral swab with clinical diagnosis of HIV/AIDS produced the mixed growth of various non-albicans species together with C. albicans and non- albicans with non- albicans.

5 Conclusion

Candida albicans (56.5%) is the most prevalence of *Candida* species isolate from clinical specimen in Ilorin, Kwara State, Nigeria in this study and *C. albicans* is also the most prevalence in Female (59.6%) while *C. tropicalis* has the highest prevalence in Male (36.8%). Age group between 20 - 29 has the highest prevalence of candidiasis (43.5%). Clinical specimens that produce highest number of *Candida* species in this study was High Vaginal Swab (58.2%), Pelvic Inflammatory Disease (PID) and Vulvovaginal candidiasis were the most clinical diagnosis (34.1%) and (28.9%) respectively, female subjects have the highest prevalence of *Candida* infections (91.8%) while males have (8.2%) in Ilorin, Nigeria.

The results of this study indicates that all the isolates of *Candida* species grew well on CHROM agar *Candida* differential medium and this is in line with the fact that this medium has good performance, good turnaround time and having sensitivity for the isolation/speciation of *Candida albicans* and nonalbicans. *Candida* isolates after 24 hours of

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incubation on CHROM agar revealed good luxuriant colours. This agrees with previous reports that almost all colonies form discrete colonies and distinct colours on CHROM agar. There were also mixed growth of *Candida* species isolated from this study which shows that CHROM agar is capable of isolating more than one strains of *Candida* species from a single clinical specimen.

6 Recommendation

- The clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility. Therefore, CHROM agar is a simple, rapid and inexpensive method with good sensitivity and specificity for, characterization, identification and speciation of *Candida* species and therefore
- recommended for routine diagnosis of *Candida* species.
- It is clear from the results of this study and review of previous works that the distribution of *Candida* species varies greatly from region to region. It is therefore important for local surveillance data to be gathered periodically in order to detect changes in the epidemiology of
- *Candida* infections especially among high-risk populations.
- CHROM agar *Candida* could not differentiate *C. albicans* from *C. dubliniensis* except with the aid of Germ Tube Test (GTT) which shows difference between *C. albicans* and *C. dubliniensis* by the appearance of constriction on the *C. dubliniensis*, hence, Germ Tube Test (GTT) is recommended for speciation of *C. dubliniensis*.

7 Limitation

Out of 254 total sample sizes 232 samples were collected due the short period of time for this study.

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