Isolation and Identification of *Candida auris* from Cutaneous Surface of Patients on Long-term Care in Afe Babalola University Multi-System Hospital, Ado-Ekiti, Nigeria

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Abstract: Nearly 150 *Candida* species have been described and are part of the microbiome on human skin, mucous membranes, the female genital tract, and the gastrointestinal tract, but only 10% of them are known to cause human diseases (candidiasis). The typical human commensal flora contains a number of species of *Candida*. Recently identified is C*andida auris*, a multidrug-resistant yeast has emerged as a prominent fungal pathogen due to its capacity to spread epidemics and invasive infections in healthcare settings. *Candida auris* infections have proven challenging to manage and treat. This study aimed at determining the prevalence of *Candida auris* on the skin surface of long-term inpatients in Afe Babalola University (ABUAD) Multisystem Hospital, Ado-Ekiti, Nigeria. The method includes the collection of skin swabs using the single swab axilla and groin composite collection method and culturing on the appropriate media for identification of the species. Antibiotic sensitivity test using the standard well diffusion method was also carried out. A total of 100 samples were collected and 85 isolates were obtained. The isolates obtained from inpatients (n=85), 52.9% (n=45) were *Candida albicans*, 4.7 % (n=4) *Candida glabrata*, 23.5% (n=20) *Aspergillus* species, 18.8% (n=20) unidentified *Candida* species and their susceptibility patterns were determined. About 18.75% (n=3) of the unidentified *Candida* species which showed resistance to all 3 classes of antimycotic agents used were suspected to be *C. auris*. In conclusion, a high percentage of patients showed significant growth of opportunistic fungi which may be harmful to immunocompromised patients. The information in this study can aid in enlightening patients about nosocomial infections.

Key word: Antifungal, *Candida auris*, Cutaneous surfaces, Drug resistance, Long term hospitalization

INTRODUCTION

Fungi are eukaryotes which digest food externally and absorb nutrients directly through their cell walls. Most fungi reproduce through spore production and have at hallus (body) which is made up of microscopic tubular cells called hyphae. Fungi are heterotrophs and, like animals, obtain their carbon and energy from other organisms. While some fungi obtain their nutrients from a living host, others obtain their nutrients from dead plants or animals (saprophytes/saprobes) or infect a living host, but kill host cells in order to obtain their nutrients (necrotrophs) Fungal diseases are referred to as mycoses (plural-"mycosis") (Madhavan *et al*., 2011). Fungal infections can be classified into four broad categories; the deep-seated or systemic mycoses, cutaneous mycoses, subcutaneous mycoses and superficial mycoses (Madhavan *et al*., 2011). *Candida* species are part of the microbiome, nearly 150 *Candida* species have been described on human skin, mucous membranes, the female genital tract, and the gastrointestinal tract (Ahmad *et al*., 2021). However, only about 10% are known to cause human infections (candidiasis) (McCarty *et al*., 2016). Several species of *Candida* including *Candida albicans, C. dublinensis, C. glabrata, C. guilliermondii, C. Lusitaniae, C. parapsilosis, C. tropicalis* can be found as part of the normal human commensal flora, especially in all sections of the gastrointestinal tract (Ali *et al.*, 2018). The most important species considered pathogenic to human are *C. albicans, C. tropicalis, C. Kruse, C. glabrata, C. lusitaniae* and *C. viswanathii* (Ali *et al*., 2018). The National Nosocomial Infections Surveillance System (NNISS) reports *Candida* species as the fourth most common nosocomial bloodstream pathogen in man

(Spampinato and Leonardi, 2013). Mortality rates have been estimated to be as high as 45% in man (Cheng *et al*., 2005). *Candida* species are well-known yeasts which cause various cutaneous and invasive infections (Steele *et al*., 2020). *Candida* species are capable of causing fungal infection in different parts of body known as candidiasis which may occur in the following major clinical forms: Cutaneous candidiasis is the infection of the skin and nails. The most common areas for this infection include inguinal folds in infants, skin folds and nail folds. The warmth and humidity of these areas of the skin allows the pathogen to thrive (Pelletier *et al*., 2005). Mucosal candidiasis occurs in people who are immuno-compromised, have poor oral hygiene, hyposalivation, dentures and smokers who have more risk to have candidiasis of mucosal membrane. Oral thrush is candidiasis of mouth, while vulvovaginal candidiasis is infection of female genital tract (Pelletier *et al*., 2005). Disseminated candidiasis can also be called invasive or systemic candidiasis. It is a serious infection that can infect blood, eyes, brain, liver and can cause disseminated disease (Pelletier *et al*., 2005). Patients who are immuno-compromised are susceptible to these infections (Koundal and Cojandaraj, 2020). However, *Candida* species were not considered a serious global health threat until the recent emergence of *Candida auris* which was first reported in the ear canal of a patient in Japan in 2009 (Steele *et al*., 2020). *Candida auris* is a multi-drug resistant, highly transmissible pathogen, therefore, a high percentage of patients infected by these fungi may lead to a disease outbreak in the hospital and possibly in the general society. This study seeks to determine the prevalence of *Candida auris* on the skin surface of long-term inpatients in ABUAD Multisystem Hospital, Ado-Ekiti, Nigeria

MATERIALS AND METHODS

Study design: This study is a hospital based cross-sectional study. Skin swabs were collected from long-term inpatients at ABUAD Multisystem Hospital, Ado-Ekiti and transported to the laboratory where culture, biochemical testing and antifungal susceptibility testing were done.

Study area: Ado-Ekiti, the study area, is located at about 48 kilometers north of Akure, Ondo state capital, about 344 kilometers north of Lagos (Nigeria) and about 750 km south-west of Abuja, the Federal Capital Territory (FCT). Ado Ekiti is the Ekiti State capital and a Local Government Headquarter in one of the sixteen Local Government Areas in Ekiti State, Nigeria. It lies within Latitude 7°10' and 7°45' north of the Equator and Longitudes 5°10' and 5°28' east of the Greenwich meridian (Owolabi, 2020).

Sample size: The sample size of 99 was calculated using the formula;

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where;

n= the minimum size required

Z=statistic for a level of confidence (1.96)

P=working proportion=6.89%=0.689 (Mbakwem-Aniebo *et al*., 2020)

D=precision (5%/100=0.05)

The working proportion (P) was determined by the prevalence of *Candida* based on other studies.

$$n = \frac{1.96^2 \times 0.0689(1-0.0689)}{0.05^2}$$

$$= \frac{3.8416 \times 0.0689(0.9311)}{0.0025}$$

$$= 98.57 \cong 100$$

Therefore, the minimum sample size for the study was estimated to be 100

Inclusion criteria: Long-term inpatients with a hospital stay of 1 week to 2 months were included in the study.

Exclusion criteria: Outpatients and recent inpatients with a hospital stay of less than 1 week were excluded from the study. Also, patients who do not give consent were excluded from the study.

Ethical consideration: Ethical approval to carry out the study was sought for and obtained from the Ethical and Research Committee of ABUAD Multi-system hospital. The nature and purpose of the

research was explained to each participant using an informed consent form for literate participants and verbal explanation for illiterate participants. Participants were not forced to participate, but at their own free will. The participants were assured of confidentiality.

Sample collection: Skin swab samples were collected from inpatients using a sterile swab with the aid of the single swab axilla and groin composite collection method.

Culture: Skin swabs were cultured on Sabouraud dextrose (SDA) agar and corn meal agar (CMA) and then incubated at 37°C for 24 hours.

Gram staining: A smear was made on clean grease free glass slide and hit fixed using a Bunsen burner. Gentian violet was used to flood the slide for one minute and rinsed with water. Lugol's iodine was used to flood the slide for one minute and rinsed with water. Acetone was used to decolorize the smear briefly and it was rinsed immediately with water. Safranin was added to counterstain the smear for one minute and it was rinsed with water and was observed with x100 lens microscope for gram stain reaction.

Germ tube test: Muller-Hinton broth, 0.5ml and 0.5 ml of fresh plasma were mixed in a sterile tube. Using a sterile wire loop, the yeast was inoculated into the mixture and incubated at 37°C for 2 hours. A drop of the incubated mixture was placed on a clean grease-free glass slide and covered with a cover slip. Microscopic examination of the mixture was done using ×10 and ×40 objectives.

Carbohydrate fermentation test: A drop of overnight broth was placed into the prepared sugars glucose, sucrose, and lactose. It was incubated overnight at 37°C and observed for color change.

Antifungal susceptibility testing using well diffusion method: The colonies were inoculated into Muller-Hinton broth using a sterile wire loop. Using the pour plate method, the colonies were inoculated in Sabouraud Dextrose Agar containing vancomycin for bacterial growth inhibition, the organisms were inoculated on the SDA plate**,** a borer was sterilized by heating with Bunsen burner and holes were made on the SDA plate. Equal volume of each antifungal agent was pipetted into respective holes and the plates were incubated for 24 hours at 37°C and the zones of inhibition were observed.

Data analysis: The data was analyses and interpretation using descriptive statistics to explained the characteristics of interest in this study. Prevalence and susceptibility of the characteristics of interests were estimated and presented in tables and charts.

RESULTS

In the present study, out of 85 total samples obtained, 35 were males and 50 were females. From the male sample, number of *Candida albicans* was 12, *Candida glabrata* was 1, *Aspergillus* species was 13 and unidentified species was 9. Among the females, 33 were *Candida albicans* 3, *Candida glabrata*, 7 *Aspergillus* species and 7 were unidentified *Candida* species (Table 1). This study shows the total prevalence of isolated *candida* species on the cutaneous surface of long-term inpatients at ABUAD Multisystem hospital and prevalence among male and female in patients (Figure 1). From the total isolates obtained from female inpatients (n=50), 66% were *Candida albicans*, 6% were *Candida glabrata*, 14% were *Aspergillus* species, 14% were unidentified *Candida* species (Table 2). This study shows the susceptibility patterns of the isolates obtained during this study; 75.56% *C. albicans* were susceptible to fluconazole, while 13.33% were intermediate and 11.11% were resistant. 75% of *C. glabrata* were susceptible, while 25% were intermediate against fluconazole. Also, 45% of *Aspergillus* spp. was susceptible, 30% were intermediate and 25% were resistant to fluconazole. 75% of unidentified *Candida* species were susceptible while 25% were resistant to fluconazole (Table 4). In the present study as shown in figure 2, 75.56% of *C. albicans* were susceptible, 8.89% were intermediate and 8.89% were resistant

against Amphotericin B. 75% of *C. glabrata* were susceptible while 25% were intermediate against amphotericin B, 35% of *Aspergillus* spp. were susceptible, 5% were intermediate and 60% were resistant against Amphotericin B. 81.25% of unidentified *Candida* species were susceptible while 18.75% were resistant against amphotericin B (Table 4). This work revealed that 75.56% *C. albicans* were susceptible, 20% were intermediate and 20% were resistant against caspofungin. 100% of *C. glabrata* were susceptible to caspofungin. The findings showed 80% of *Aspergillus* spp. were susceptible while 20% were resistant against caspofungin. 62.5% of unidentified *Candida* species were susceptible, 12.5% were intermediate, while 62.5% were resistant against caspofungin (Figure 3) (Table 4).

Table 1: Gender distribution of fungal isolates

Organism Isolated	Number (Male)	Prevalence (Male)	Number (Female)	Prevalence (Female)	Total	Total %
Candida albicans	12	34.3	33	66	45	52.9
Candida glabrata	1	2.9	3	6	4	4.7
Aspergillus species	13	37.1	7	14	20	23.5
Unidentified *Candida* species	9	25.7	7	14	16	18.8
Total Isolates	35		50		85	

Table 2: Prevalence of isolated organisms among male and female inpatients

Organism Isolated	Number (Male)	Number (Female)	Total	Prevalence (Male) %	Prevalence (Female) %	Total Prevalence (%)
Candida albicans	12	33	45	34.29	66	52.94
Candida glabrata	1	3	4	2.86	6	4.71
Aspergillus species	13	7	20	37.14	14	23.53
Unidentified *Candida* species	9	7	16	25.71	14	18.82
Total Isolates	35	50	85			

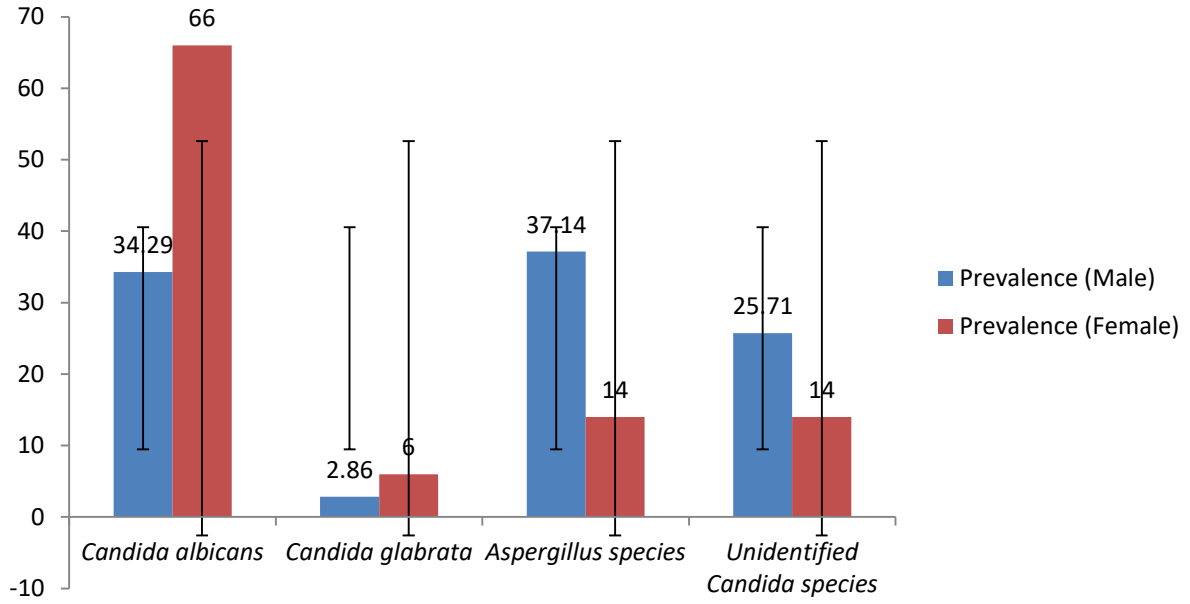

Figure 1: Prevalence of fungal isolates in relation to gender of participants

Table 3: Antimycotic susceptibility patterns of fungal isolates

Antifungal agent	Isolate	Total	Susceptibility patterns of isolates		
			Susceptible	Intermediate	Resistant
Fluconazole	*Candida albicans*	45	34	6	5
	Candida glabrata	4	3	1	-
	Aspergillus species	20	9	6	5
	Unidentified *Candida* species	16	12		4
	Candida albicans	45	37	4	4
Amphotericin B	*Candida glabrata*	4	3	1	-
	Aspergillus species	20	7	1	12
	Unidentified *Candida* species	16	13	-	3
Caspofungin	*Candida albicans*	45	27	9	9
	Candida glabrata	4	4	-	-
	Aspergillus species	20	16	-	4
	Unidentified *Candida* species	16	10	2	4

Table 4: Profile of the antimycotic susceptibility patterns of fungal isolates to fluconazole, amphotericin B and caspofungin

Antifungal agent	Isolate	Total number	Susceptibility patterns		
			Susceptible %	Intermediate %	Resistant %
Fluconazole	*Candida albicans*	45	75.56	13.33	11.11
	Candida glabrata	4	75	25	-
	Aspergillus species	20	45	30	25
	Unidentified *Candida* species	16	75	-	25
Amphotericin B	*Candida albicans*	45	82.22	8.89	8.89
	Candida glabrata	4	75	25	-
	Aspergillus species	20	35	5	60
	Unidentified *Candida* species	16	81.25	-	18.75
caspofungin	*Candida albicans*	45	60	20	20
	Candida glabrata	4	100	-	-
	Aspergillus species	20	80	-	20
	Unidentified *Candida* species	16	62.5	12.5	25

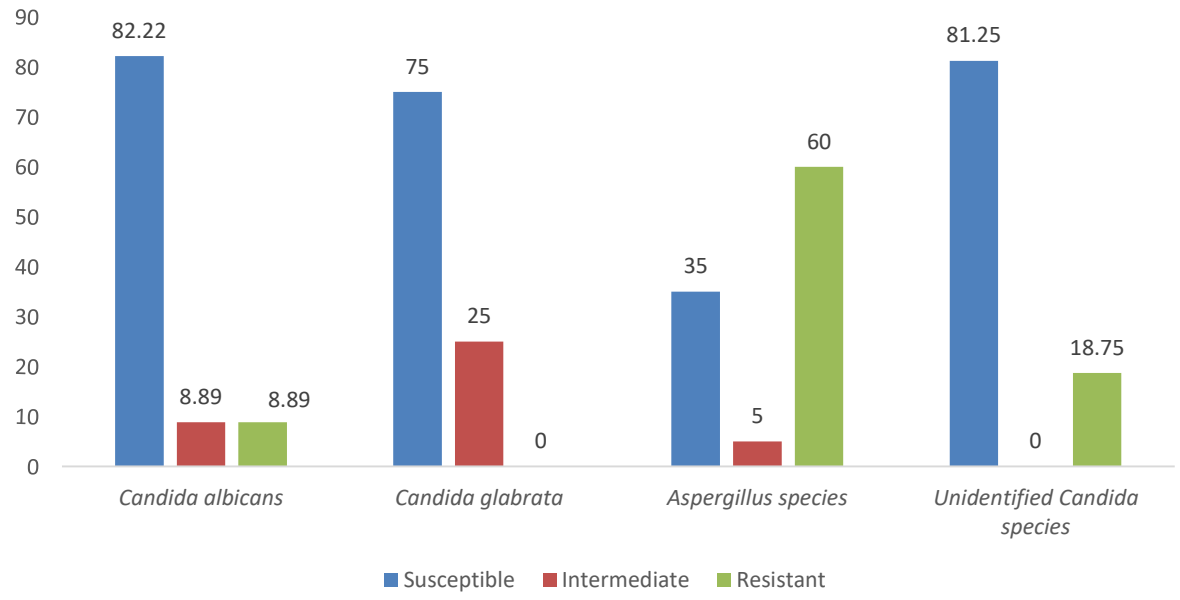

Figure 2: Susceptibility patterns of fungal isolates to amphotericin B

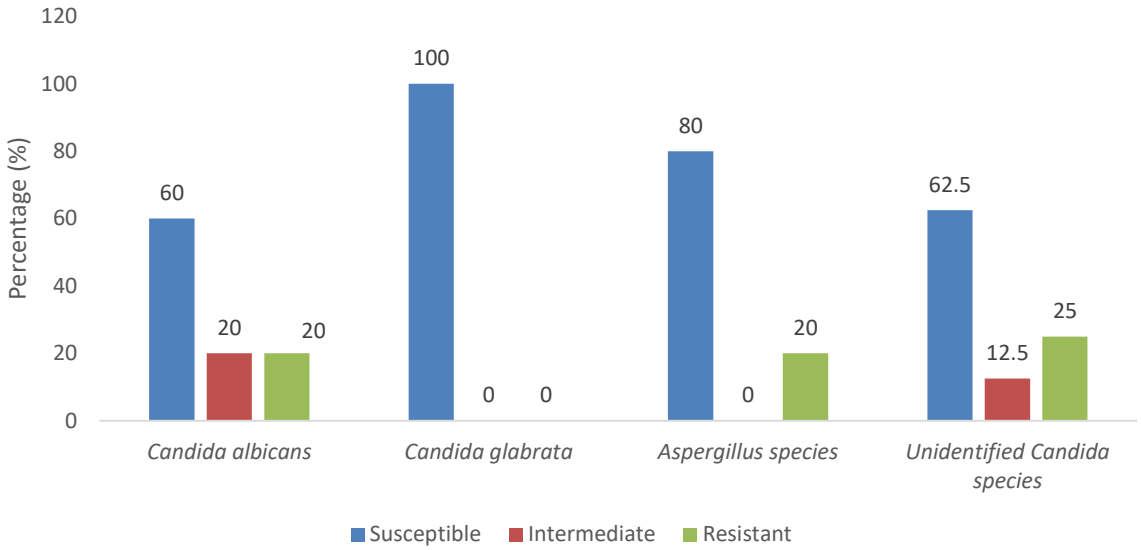

Figure 3: The susceptibility patterns of fungal isolates to caspofungin

DISCUSSION

Candida auris poses a severe threat to global health as it spreads readily in healthcare facilities and can cause serious illnesses (CDC, 2019). Over a billion individuals are affected by and over 1.5 million individuals die from fungal diseases (Bongomin *et al*., 2017). Though the majority of deaths from fungal illnesses are preventable, they are nonetheless an issue that public health authorities ignore (Bongomin *et al*., 2017). More than 150 million individuals have serious fungal diseases that have a significant impact on their lives or are fatal. Nearly a billion people are believed to have fungal infections of the skin, nails, and hair (Bongomin *et al*., 2017). Mucosal candidiasis affects many millions of people (Bongomin *et al*., 2017). The severity, however, can range from asymptomatic mild mucocutaneous infections to systemic infections that could be fatal (Bongomin *et al*., 2017).

In this study, a total of 100 samples were collected from the skin of inpatients at ABUAD Multisystem hospital, cultured and 85 isolated were obtained. Out of the 85 isolates obtained, 52.93% were *Candida albicans*, 4.71% were *Candida glabrata*, 23.5% were *Aspergillus* species and 8.82 were unidentified yeast cells. After *Candida albicans*, *Aspergillus* species is the most frequent opportunistic fungal infection in humans (Tahir *et al*., 2011). In immunocompromised people, it leads to serious infections that have a high fatality rate, particularly in new-borns (Tahir *et al*., 2011). For a good length of time, *Candida* species have co-existed as the most prevalent and benign commensals linked with humans (Mahalingam *et al*., 2022). *Candida* spp frequently appear on human skin (Mahalingam *et al*., 2022). However, *Candida* spp develop into opportunistic pathogens in immunologically weakened, preterm infants, aged, and immunocompromised persons (Mahalingam *et al*., 2022). Candida's pathogenic adaptations present as localized mucosal infections or systemic infections, with the potential to occasionally spread to important organs (Mahalingam *et al*., 2022). The genital area in men and women is a frequent site of *Candida* infection. Although there are several antifungal medications available, not all of them work against *Candida* (Pappas *et al*., 2016). The problem with limited effectiveness in therapy is not the only one. *Candida* species are among the pathogens which are becoming more resistant to

antifungal medications presently (Talapko *et al*., 2021).

This study showed a high level of *Candida albicans* compared to other organisms isolated. In 50% of the population, *C. albicans* is part of the normal flora of the microbiota and around 70% of fungal infections worldwide are caused by *C. albicans*, which is also the most frequent cause of mucosal infections and systemic infections (Talapko *et al*., 2021).This study showed that a high percentage of opportunistic fungi which have significant resistance to antimycotic drugs (fluconazole, amphotericin B and caspofungin) are present on the skin of patients in ABUAD multisystem hospital. Such opportunistic fungi may pose various levels of threats to the health of immunocompromised patients.

CONCLUSION

From the result obtained, 18.75% (n=3) unidentified *Candida* species isolates which showed multi-drug resistance to all 3 classes of antifungal agents used were suspected to be *Candida auris*. However, further testing of the isolates using molecular techniques is required to fully identify these isolates. The advent of *C. auris* introduces a fresh threat to global healthcare. Although there are numerous descriptions of outbreaks and this novel pathogen has been the subject of systematic analyses which have shed some light on the specifics of its emergence and the best control strategies, solid evidence for clinically effective interventions is still insufficient.

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