Vol. 1 No.1, July 2021

FUAM Journal of Pure and Applied Science

Available online at
www.fuamjpas.org.ng

An official Publication of
College of Science
Joseph Sarwuan Tarka University,
Makurdi.

FUAM Journal of Pure and Applied Science
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https://fuamjpas.org.ng/
Vol.1. No. 1. July, 2021

Antifungal resistance of Candida albicans isolated from high vaginal swab of students attending Joseph Sarwuan Tarka University Clinic, Makurdi, Benue State, Nigeria.

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Received: 19/07/2021 **Accepted**: 13/08/2021 **Published online**: 16/08/2021

Abstract
This study was carried out to determine the antifungal resistance of *Candida* isolated from female students attending the school Clinic. A total of 500 high vaginal swab samples were collected using swab stick. Consent forms were also administered to the female students whose samples were collected. Isolation and identification of the *Candida* species was carried out by microscopy, biochemical test, Germ-tube test and use of fungal identification kits. Antifungal susceptibly testing was performed by disk diffusion method using Itraconazole, Ketoconazole, Nystatin and Griseofulvin. Statistical package for social sciences was used as a tool for data analysis. *Candida albicans* (80.9%) and *Candida glabrata* (19.1%) were isolated from the samples. Antifungal resistance of the isolates were also determined, where *C. albicans* had 100% resistance to Griseofulvin, 80% to itraconazole and nystatin and 40% to ketoconazole. While *C. glabrata* had 100% resistance to Giseofulvin, nystatin and itraconazole and 40% to ketoconazole. *C. albicans* and *C. glabrata* are the common cause of vaginal candidiasis among the female students and ketoconazole, may be a better drug for the treatment of this infection.

Keywords: Antifungal, *Candida albicans*, HVS, Germ-tube, RapID Yeast Plus System

Introduction

superficial mucosa lesions to invasive, life threatening disease [1]. Among these species, *C. albicans* is most abundant and exist as harmless commensal in the gastrointestinal and genitourinary tracts in about 70% of humans [18]. However, it becomes opportunistic pathogen for immunocompromised patients [29,35,39], for some immunologically weak individuals or even healthy persons [21,32]. The infection caused by *C. albicans* as well as non-*Candida albicans* is called candidiasis [14,37]. It has been reported that about 50% of worldwide candidemia cases are caused by *Candida albicans* [1,7, 19]. Just about 20 years ago that there is an increase in cases of non- *albicans* species [7,10]. *Candida* posses some traits that promote its pathogenicity, these traits includes phenotypic switching [2], yeast-hyphae transition [12], secretion of molecules that promote adhesion to surfaces [4] and presence of point mutation [21,42,43]. *Candida* species have to maintain a balance between its ability to invade host tissues and the host defense mechanism [13,15,20]. Alteration of this delicate host-fungus balance can result in high levels of patient mortality [5,28] systemic *Candida* infections are fatal in 42% of cases [41], despite the use of antifungal therapies [31,33,34,36]. *Candida* infection is the most common infection in hospital [8,20,9,22,3923,24]. The numerous cases of *Candida* infection today is as a result of its resistance to available or commonly used antifungal agents or drugs [25,26,27,40].

The incidence of invasive fungal infections poses a serious health threat globally, killing a lot of people every year. However, the expanding use of antifungal drugs on patient is associated with increasing incidence of Antifungal drug resistance resulting from inherently less sensitive *Candida* species. Therefore, this study centers on comparing the resistance of two widely used antifungal drugs; Azole and Polyenes.

This study provides information on the drugs with high activity on *Candida* species, which is hoped to guide health administrators in treating patients suffering from *Candida* infection.

Materials and Methods

Study area

This research was carried out in Benue State located on latitude $7^0$34N and longitude $8^0$34E.

Ethical approval

The Ethical approval for this study was obtained from the Ethical Committee on Research of Infectious Diseases of Federal Medical Center, Makurdi, Benue State. Consent was also obtained from the female students that presented themselves for medical treatment in the University clinic before sample collection. The approval was on the agreement that participants' anonymity will be maintained, good laboratory practice/quality / control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. However, female students that desire to know the results of antifungal susceptibility testing would be given (verbally) free of charge.

Sample collection

Samples used in this study were collected from female students of Joseph Sarwauan Tarka University clinic, Makurdi. A total of 500 samples were collected in June, 2019. Samples were collected using sterile swab sticks from consented patients which attended the University clinic. Sampling was assisted by specialist medical doctors who were given consent form to administer to patients.

Culture media

Sabouraud dextrose agar (SDA): This medium was used for the isolation of Candida species. Mueller Hinton agar (MHA): This was used for antifungal susceptibility testing [6].

Preparation of culture media

All media used were prepared according to manufacturer's instructions. Sabouraud dextrose agar (7.9g) was suspended in 250mL of distilled water, mixed well until uniform suspension was obtained and heated at 121◦C for 15 minutes. The agar was allowed to cool at room temperature, an antibiotic (Pefloxacine) was added to inhibit bacteria growth and 20mL of the agar was poured into each of the petri dishes.[1,6]

Inoculation of samples

The swab sticks were streaked directly on the surface of the agar in three parallel lines to point A, then to B and point C, with a zigzag line at the middle and incubated at 37^0C for 72 hours [1].

Isolation and identification of Candida species.

Candida species were isolated and identified based on microscopy, biochemical test, germ tube and fungal identification kit (RapID kit) [1].

Microscopy

About 1ml of normal saline was put in the tube containing the swab stick and covered cotton wool, shaken and allowed to stand for some minutes. A drop of the saline was made on a clean grease free slide and viewed with X10 and then X40 objective lens. It shows a budding yeast with pseudohyphae [1,6].

Biochemical test.

Rapid yeast plus system was used to identify Candida species. Pure culture of the yeast was inoculated into the rapid yeast plus system panel containing fungi food substrates and enzymes for 4hours. Following the manufacturer's instructions, Antigen A and Antigen B were added to their corresponding wells on the panel and further reactions were observed and matched with the manufacturers color chart [1].

Germ tube test

Germ tube test as a method of identification of *Candida albicans* was also carried out on suspected yeast colonies. Using a sterile wire loop small portion of the colonies were inoculated into peptone water for 24 hours and incubated at 37°C to obtain a pure culture. The pure culture was inoculated in sterile test tubes containing 0.5mL of human serum which was incubated for 4 hours. A drop of the mixture was placed on a grease free slide with a drop of lactophenol blue stain and covered with a cover slip. It was then examined microscopically at X10 and X40 objective lenses. [17]. *Candida albicans* formed germ tubes with short lateral hyphal filaments without any constrictions.

Use of identification kits

The isolated samples were grown on Sabouraud Dextrose Agar. The inoculum was prepared by picking 4 well isolated colonies and making a homogenous suspension in 3mL sterile saline. The density of the suspension was adjusted to 0.5OD at 620 nm. The Identification Kit was opened aseptically. The sealing foil was peeled off. Each well was inoculated with 50µl of the above inoculum by surface inoculation method as prescribed by the manufacturer.

Antifungal sensitivity testing

Antifungal susceptibility testing was performed by four antifungal drugs disk; Itraconazole, Nystatin, Ketoconazole and Griseofulvin. Antifungal susceptibility testing was performed by disk diffusion method using Mueller-Hinton Agar 2% Glucose and 0.5 ug/mL Methylene Blue Dye (GMB) medium as per clinical and laboratory standard institute [6] 0.5 McFarland standard was used to

standardize the inoculum density. The fungal susceptibility of the isolates was interpreted in percentages.

Results and Discussion

Table 1 shows the result of microbiological analysis in relation to the distribution of vaginal candidiasis among female students. The macroscopy, microscopy and identification tests were able to detect *Candida albicans* as the highest distributing specie with a distribution of 80.9% and *Candida glabrata* has a distribution of 19.1%.

Table 1: Microbiological analysis in relation to the distribution of *vaginal candidiasis*.

Macroscopy	Microscopy	Biochemical Test	Inference	No (Distribution) (n=21)
Smooth, creamy and pasty colored colonies	Oval shaped single budded cells with pseudohyphae.	RAPID yeast plus system	*Candida albicans*	17 (80.9%)
Smooth, creamy and pasty colored colonies	Germ tube positive without constriction Oval shaped single budded cells with pseudohyphae. Germ tube negative	RAPID yeast plus System	*Candida glabrata*	4 (19.1%)

Table 2 shows Antifungal Resistance of *Candida* isolated from female students with suspected cases of vaginal candidiasis attending Joseph Sarwuan Tarka University Clinic, Makurdi, in percentages. Candida albicans is 100% resistance to Griseofluvin (AGF), while Nystatin and Intraconazole had 80% resistance whereas Ketoconazole had the least resistance of 40%. *Candida glabrata* shows 100% resistance to Griseofluvin, Nystatin, and Itraconazole respectively, and it is 80% resistance to Ketoconazole.

Table 2 Antifungal resistance of *Candida* isolated from female students with suspected cases of *vaginal candidiasis* attending Joseph Sarwuan Tarka University Clinic, Makurdi.

Isolates	No. Resistance (%)			
	KCA	AGF	NY	ITC
Candida albicans	2(40)	5(100)	4(80)	4(80)
Candida glabrata	4(80)	5(100)	5(100)	5(100)

Key: AGF = Griseofluvin, NY = Nystatin, ITC = Intraconazole, KTC = Ketoconazole

Table 3 zones of inhibition on Candida isolated from students with suspected cases of vaginal candidiasis. Griseofluvin has the highest number of resistance isolates to be 100% resistance, followed by itraconazole and Nystatin to 80% respectively and lastly Ketoconazole which have 40% resistance. This table also contains Antifungal break point with Ketoconazole and itraconazole having the highest breakpoint of ≥19 respectively followed by Nystatin ≥17 and lastly Griseofluvin ≥16.

From the data above the *Candida* species isolated from female students are *Candida albicans* and *Candida glabrata*. Twenty of these isolates (five each) were subjected to antifungal susceptibility testing to two azoles (ketoconazole and itraconazole) and two polyenes (nystatin and griseofulvin). These isolates had 100% resistance to griseofulvin, 80% resistance to nystatin and itraconazole and 40% resistance to ketoconazole respectively.

Table 3 Antifungal zones of inhibition on *Candida* isolated from female students attending Joseph Sarnwuan Tarka University Clinic, Makurdi, with suspected cases of vaginal candidiasis.

Antifungal drugs	Disc content(ug)	Antifungal break point(mm)	No. of isolates	No. of resistance isolates (%)	MIZD (mm) (±SD)	IZDR (mm)	F	Sig
KCA	10	≥19	5	2(40)	1.780±5.341	6.00-20.00	29.094	0.001
NY	100	≥17	5	4(80)				
AGF	10	≥16	5	5(100)				
ITC	08	≥19	5	4(80)				

This research work was carried out to isolate *Candida* species associated with vaginal candidiasis, present in the HVS samples got from the female students attending Joseph Sarnwuan Tarka University Clinic, Makurdi, after which the isolates were subjected to Antifungal susceptibility testing.

The *Candida* species isolated are *Candida albicans* and *Candida glabrata,* had 80.9% and 19.1% respectively, which agree with the findings of [16,17] that *C. albicans* are the most abundant *Candida* species, although, there has being a rising trend of recovery of new non albicans species. Possession of traits that promotes pathogenicity of *Candida* isolates could account for *C. albicans* 80.9% as reported by [4,12 21]. Other factors such as high sexual activities as students (undergraduates) are within the age range of 16-26, indulge in unprotected and indiscriminate sex. Also, abuse of antibiotics/antifungal agents, wearing of air tight under wears and sharing of under wears with other students, hormonal imbalance due to growth development and pregnancy, predispose the students to candidiasis [1,17]. The study also reveals that the use of antibiotics in the treatment of bacterial infections, which not only kill the pathogenic bacteria but also kill the healthy vagina bacterium (lactobacillus) thereby predisposing these female students to vaginal candidiasis [17,38].

Both *Candida albicans* and *Candida glabrata* are highly resistant (100% resistant) to Griseofulvin. This is because Griseofulvin's spectrum of activity is limited to dermatophytes which possess a prolong energy-dependent transport system for the antifungal drug as reported by [3].

Nystatin and Itraconazole had 80% resistance to *C. albicans* and *C. glabrata*, this study disagree with the studies done by [30] which showed that identification of polyene resistant *Candida* isolates are difficult to reproduce and that filamentous fungi are more likely than yeast to have reduced susceptibility to polyenes as *C. albicans* and *C. glabrata* being yeast but show reduced susceptibility to polyenes. Ketoconazole has 40% resistance and this implies that *C. albicans* and *C. glabrata* are highly susceptible to Ketoconazole, although it has limited spectrum of activity this is similar to the studies carried out by [11], which said that ketoconazole is used in the treatment of superficial and deep fungal infections. The azoles had higher activity against *Candida* isolates than the polyenes.

Conclusion

Candida albicans and *Candida glabrata* are the common cause of vaginal candidiasis among the female students attending the school clinic. These isolates show high resistance to Griseofulvin followed by Nystatin, Itraconazole. In addition, the class of antifungal drugs that is required for the treatment of vaginal candidiasis among the female students is Keteconazole (Azole), hence *Candida* shows reduced resistance to it.

Declaration of conflicting interests

The authors declared no potential conflicts of interest

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Cite this article

Agada E.O., Pinen A. and Aeanan P.T. (2021). Antifungal resistance of *Candida albicans* isolated from high vaginal swab of students attending Joseph Sarwuan Tarka University Clinic, Makurdi. Benue State, Nigeria. *FUAM Journal of Pure and Applied Science*, **1**(**1**):81-87