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Antifungal Resistance Pattern of *Candida* Species Isolated from High Vaginal Swabs of Women Attending a Hospital in Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JOE and IO designed the study, while author JOE wrote the protocol, did the literature searches and wrote the manuscript. Authors CAO and IO supervised the entire study. The three authors managed the analysis of the study, read and approved the final manuscript.

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ABSTRACT

There is an increase in non-albicans *Candida* (NAC) vulvovaginal candidiasis which is attributed to overuse of antifungal therapy and this has led to antifungal resistance. This study was aimed at determining the antifungal resistance pattern of some clinical isolates of *Candida* species. Eighty-eight (88) isolates were used which included *Candida tropicalis* (34), *Candida Parapsilosis* (21), *Candida albicans* (20), *Candida krusei* (7) and *Candida glabrata* (6). The drugs used were Fluconazole (25µg), Ketoconazole (10µg), Voriconazole (1µg), Nystatin (100Units), Amphotericin B (20µg), Flucytosine (1µg), Clotrimazole (10µg) and Itraconazole (50µg). The susceptibility testing was carried out using the M44-A standard method for yeast disk diffusion testing. Results showed that the percentages of *Candida* species resistant to Fluconazole, Ketoconazole, Voriconazole, Amphotericin B, Flucytosine, Clotrimazole and Itraconazole and Nystatin were 52.3%, 61.9%, 35.2%, 19.3%, 86.4%, 34.1%, 45.5% and 44.3%, with inhibition zone diameters ≤14mm, ≤20mm, ≤13mm, <10mm, ≤11mm, ≤11mm, ≤13mm and no inhibition zone diameter respectively. *Candida krusei* was the most resistant species with 100% resistance to each of Fluconazole, Ketoconazole

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and Flucytosine. *Candida tropicalis* was the species with the highest susceptibility (79.4%) to Amphotericin B followed by *Candida parapsilosis* with inhibition zone diameters ≥15mm. While *Candida glabrata* showed 100% resistance to each of Flucytosine and Itraconazole, *Candida albicans* showed 100% resistance to Flucytosine only. *Candida glabrata* was the only *Candida* species with 0% resistance to Amphotericin B. The drug to which most of the *Candida* species were susceptible was Amphotericin B followed by Voriconazole while Flucytosine was the drug with the highest resistance followed by Ketoconazole and Fluconazole. The highest number of susceptible-dose dependent *Candida* isolates was observed with Ketoconazole (25%), followed by Clotrimazole and Itraconazole, each recording 23.9%. Based on the findings of the present study, Voriconazole is recommended for vaginal candidiasis especially in the study area and also especially for infections caused by Fluconazole-resistant *Candida* species. This suggests that routine sensitivity testing is pertinent to guiding the choice of antifungal therapy. Thus, indiscriminate use of antifungal drugs should be avoided to reduce the development and spread of resistance.

Keywords: Resistance; Candida species; Vulvovaginal candidiasis; antifungal drugs; Enugu state.

1. INTRODUCTION

Serious fungal infections afflict millions of patients annually resulting in more than 1,350,000 deaths. The most serious fungal infections occur as a consequence of other serious health problems such as asthma, AIDS, cancer, and organ transplantation, and they all require antifungal therapy for a successful outcome. Failure to treat effectively either because of diagnostic delays or missed diagnosis often leads to death or serious illness. This recognition has resulted in a significant increase in antifungal agents use for the treatment and prevention of fungal infections. Yet, therapeutic options are limited; as the most widely used antifungal drugs comprise only a few chemical classes including azoles, polyenes, and echinocandins [1,2].

Candida species are well known for causing infections in mouth, skin, and vagina in humans [3]. The second most common cause of abnormal discharge after bacterial vaginosis in healthy women of reproductive age is vulvovaginal candidiasis [4]. Some studies have reported that three fourth (75%) of women will experience an episode of vulvovaginal candidiasis in their lifetimes, 50% of these will experience at least a second episode, and 5–10% of all women experience recurrent vulvovaginal candidiasis [5,6,7]. *Candida albicans* is the most common cause of vulvovaginal candidiasis, although the frequency of vulvovaginal candidiasis caused by other *Candida* species, such as *C. tropicalis*, *C. glabrata*, and *C. krusei* is increasing [8].

Candidiasis is a fungal infection caused by the yeast *Candida*. *Candida* can cause infections if it grows out of control or if it enters deep into the body (for example, the bloodstream or internal organs like the kidney, heart, or brain). Some types of *Candida* are resistant to the antifungals used to treat them [2,9]. Antimicrobial resistance occurs naturally over time, usually through genetic changes. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. However, the misuse and overuse of antimicrobials is accelerating this process [10,11].

Although *Candida albicans*, which causes most *Candida* infections in people has very low levels of drug resistance, other types of *Candida,* are frequently resistant and more deadly [3]. Yeast infections resistant to antifungal agents have been increasing and their frequency will likely continue to increase [8].

In South Eastern Nigeria, little is known about the distribution and antifungal resistance pattern of *Candida* species isolated from clinical samples, high vaginal swabs inclusive. Most often, *in vitro* susceptibility testing is used most importantly to detect resistance as well as to select agents with likely activity for a given infection. Many clinical laboratories do not have the capacity to test *Candida* for drug resistance, limiting the ability to guide treatment and track resistance [3]. [8] Also noted that lack of expertise in the field can also be incriminated as a factor. This study was, therefore aimed at determining the antifungal resistance pattern of *Candida* species isolated from high vaginal swabs of women attending a hospital in Enugu State, South East Nigeria to a host of antifungal drugs.

2. MATERIALS AND METHODS

2.1 Source of Test Microorganisms

The test microorganisms were isolated from high vaginal swab (HVS) specimens of women attending the Obstetrics and Gynecology Unit of the University of Nigeria Teaching Hospital (UNTH) Ituku/Ozalla, Enugu State, Nigeria as described [12]. These organisms included *Candida tropicalis* (34), *Candida Parapsilosis* (21), *Candida albicans* (20), *Candida krusei* (7) and *Candida glabrata* (6).In all, there were eighty-eight (88) yeast microorganisms.

2.2 Standardization of Inoculum and *in vitro* Antifungal Susceptibility Testing Using Commercial Antifungal Discs

Using a sterile wire loop, discrete colonies each of 24 hours pure culture of the *Candida* isolates was picked and inoculated into 5ml of sterile 0.85% saline. The turbidity of the suspension was adjusted and then matched visually with 0.5 McFarland standards which is equivalent to 1 x 10^6 colony forming units per ml (CFU/ml). The antifungal susceptibility testing was carried out using one of four standard methods for antifungal susceptibility testing, that is M44-A for yeast disk diffusion testing [13] released by the Clinical and Laboratory Standards Institute (CLSI), formerly the NCCLS (National Committee on Clinical Laboratory Standards). Eight antifungal drugs were used: Fluconazole (25µg), Ketoconazole (10µg), Voriconazole (1µg), Nystatin (100Units), Amphotericin B (20µg), Flucytosine (1µg), Clotrimazole (10µg) and Itraconazole (50µg) (Oxoid, UK and Abtek, Liverpool). Mueller Hinton Agar (TM MEDIA, TITAN BIOTECH LTD, Rajasthan, India) was prepared according to the manufacturer's instructions and poured into Petri dishes (plates). Each plate was seeded with 0.2ml of the standardized inoculum and spread plated evenly on the surface of the agar. The above antifungal discs were then aseptically placed (using sterile forceps) on the surface of the agar plates by pressing each disc down firmly to ensure complete, level contact with the agar. The plates were left for 30 minutes at room temperature on the laboratory bench for pre-diffusion and then incubated in an inverted position at 30°C for 24 hours. After the incubation period, the inhibition zone diameter was measured and recorded in millimeter (mm) using a transparent ruler [14]. The antifungal susceptibility of the isolates was interpreted as susceptible (S), Susceptible Dose-Dependent (SDD) or Intermediate (I) and Resistant (R). The results were interpreted in line with the Clinical and Laboratory Standards Institute guidelines [14] and [15].

3. RESULTS AND DISCUSSION

3.1 *In vitro* Susceptibility Profile of the *Candida* Species to Fluconazole

It was observed in this study that 27 (30.7%) of the *Candida* isolates were susceptible to Fluconazole, 15 (17%) were susceptible dose-dependent and 46 (52.3%) were resistant. *Candida glabrata* was the most susceptible (50%) followed by *Candida parapsilosis* (42.9%). Meanwhile, the highest resistance (85.7%) was shown by *Candida krusei*. In fact, none of the *Candida krusei* isolate was susceptible to Fluconazole (Table 1). This resistance (52.3%) is very much higher than the 2.6% reported by [16] in Korea. Also, the 25%, 29.4%, 42.9% and 50% susceptibilities respectively observed for *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and *Candida glabrata* does not agree with the > 90% respectively observed for *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and 84.3% for *Candida glabrata* by [17] in Kuala Lumpur, Malaysia. The result of 17% susceptible dose-dependent isolates recorded in this study is similar to 13.8% susceptible dose-dependent isolates previously recorded [16]. Also, the 0% susceptibility of *Candida krusei* to Fluconazole observed in the present study agrees with the 0% susceptibility previously reported [16]. Similarly, [18] reported 100% resistance of *Candida krusei* to Fluconazole. Isolates of *Candida krusei* are considered resistant to Fluconazole irrespective of the MIC [16]. This calls for identification of the particular etiologic agent (*Candida* species) and sensitivity testing to avoid ineffective and inappropriate therapy.

3.2 *In vitro* Susceptibility Profile of the *Candida* Species to Ketoconazole

Twelve (13.6%) out of the 88 *Candida* isolates were susceptible, 22 (25%) were susceptible dose-dependent and 54 (61.4%) were resistant. *Candida krusei* was the most resistant (71.4%) followed by *Candida albicans* (70%) while *Candida parapsilosis* was the most susceptible dose-dependent species (Table 2). *Candida albicans* had a 15% susceptibility to

ketoconazole which is very much lower than the 73% susceptibility reported by [19]. [19] also reported a 33.55% resistance of *Candida krusei* against ketoconazole which is lower than the 71.4% resistance observed in the present study. However, [20] reported a high resistance (83.3%) for *Candida krusei* against ketoconazole which is similar to the high resistance (71.4%) observed in the present study.

3.3 *In vitro* Susceptibility Profile of the *Candida* Species to Clotrimazole

From the *in vitro* susceptibility profile of the *Candida* species to Clotrimazole (Table 3), it was observed that 37(42.0%) of the *Candida* isolates were susceptible, 21 (23.9%) were susceptible dose-dependent while 30 (34.1%) were resistant. The highest resistance (71.4%) was observed with *Candida krusei* while *Candida parapsilosis* was the most susceptible (57.1%). *Candida tropicalis* was the most susceptible dose-dependent species (32.4%) and also the least resistant species (26.5%). *Candida albicans* had 30% susceptibility and 40% resistance to Clotrimazole (Table 3). [21] Reported 36.2% sensitivity and 63.8% resistance of *Candida albicans* against Clotrimazole. In a similar study carried out in Northwest Ethiopia, [22] reported that of the 96 *Candida* isolates tested against Clotrimazole, only 7 (7.3%) were resistant. This is very much lower than the 34.1% resistance recorded in the present study. [22] Reported a much higher susceptibility (77.2%) of *Candida albicans* in their study. The 66.7% resistance of *Candida tropicalis* against Clotrimazole reported by [21] in Jos, North Central Nigeria is much higher than the 26.5% resistance recorded in the present study. However, [21] reported 75.4% resistance against Clotrimazole by *Candida krusei* which is similar to the 71.4% observed in the present study.

Table 1. *In vitro* susceptibility profile of the *Candida* Species to Fluconazole (25µg)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	5(25)	3(15)	12(60)
Candida tropicalis	34	10(29.4)	8(23.5)	16(47.1)
Candida parapsilosis	21	9(42.9)	2(9.5)	10(47.6)
Candida krusei	7	0(0)	1(14.3)	6(85.7)
Candida glabrata	6	3(50)	1(16.7)	2(33.3)
Total	**88**	**27(30.7)**	**15(17.0)**	**46(52.3)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

Table 2. *In vitro* susceptibility profile of the *Candida* Species to Ketoconazole (10µg)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	3(15)	3(15)	14(70)
Candida tropicalis	34	5(14.7)	8(23.5)	21(61.8)
Candida parapsilosis	21	3(14.3)	8(38.1)	10(47.6)
Candida krusei	7	0(0)	2(28.6)	5(71.4)
Candida glabrata	6	1(16.7)	1(16.7)	4(66.7)
Total	**88**	**12(13.6)**	**22(25.0)**	**54(61.9)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

Table 3. *In vitro* Susceptibility Profile of the *Candida* Species to Clotrimazole (10µg)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	6(30.0)	6(30.0)	8(40.0)
Candida tropicalis	34	14(41.2)	11(32.4)	9(26.5)
Candida parapsilosis	21	12(57.1)	3(14.3)	6(28.6)
Candida krusei	7	2(28.6)	0(0.0)	5(71.4)
Candida glabrata	6	3(50.0)	1(16.7)	2(33.3)
Total	**88**	**37(42.0)**	**21(23.9)**	**30(34.1)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

3.4 *In vitro* Susceptibility Profile of the *Candida* Species to Amphotericin B

Susceptible isolates were 61(69.3%) while 10 (11.4%) and 17 (19.3%) were susceptible dose-dependent and resistant respectively. The species that was most resistant to Amphotericin B was *Candida krusei* (42.9%) followed by *Candida albicans* (25.0%). *Candida tropicalis* was the most susceptible (79.4%) followed by *Candida parapsilosis* (76.2%). There were no susceptible dose-dependent and resistant isolates of *Candida parapsilosis* and *Candida glabrata* respectively (Table 4). [23] reported a much lower resistance (15.75%) of *Candida krusei* against Amphotericin B. *Candida tropicalis* was the most susceptible (79.4%) followed by *Candida parapsilosis* (76.2%). [24] Reported 100% susceptibility of *Candida albicans*, *Candida tropicalis, Candida parapsilosis* and *Candida glabrata* to Amphotericin B. There were no susceptible dose-dependent isolates of *Candida parapsilosis* while *Candida glabrata* showed no resistance to Amphotericin B (Table 4). This agrees with the findings of [17] who reported there were no susceptible dose-dependent isolates of *Candida parapsilosis* and also 0% resistance of *Candida glabrata* against Amphotericin B. Other researchers have also documented 0% resistance of *Candida glabrata* against Amphotericin B [20,19].

3.5 *In vitro* Susceptibility Profile of the *Candida* Species to Flucytosine

The *in vitro* susceptibility profile of the *Candida* isolates to Flucytosine is shown in Table 5. Seventy-six (86.4%) of all the isolates were resistant, 6 (6.8%) were intermediate and also 6 (6.8%) were susceptible. The interpretive categories for Flucytosine are the same categories used to interpret bacterial testing. These categories include susceptible (S), intermediate (I), and resistant (R), with Intermediate (I) being substituted for the susceptible dose- dependent category. All the isolates (100%) of *Candida albicans*, *Candida krusei* and *Candida glabrata* were resistant to Flucytosine. [25] reported 80% resistance of *Candida albicans* in the United Kingdom. The method used to determine the susceptibility of the isolates can influence the results. It has been suggested that the disk method is a sensitive but not necessarily specific method to determine Flucytosine susceptibility of *Candida albicans* [25]. A very low susceptibility of 4% and 2% was observed with *Candida tropicalis* and *Candida parapsilosis* respectively. Only 6 isolates of *Candida tropicalis* (17.6%) were susceptible dose-dependent.

3.6 *In vitro* Susceptibility Profile of the *Candida* Species to Voriconazole

For Voriconazole, 46 (52.3%) of the *Candida* isolates were susceptible, 11 (12.5%) were susceptible dose-dependent and 31 (35.2%) were resistant. *Candida parapsilosis* isolates were the most susceptible (66.7%) followed by *Candida krusei* (57.1%) and *Candida tropicalis* (52.9%). There were no susceptible dose-dependent isolates of *Candida krusei* (Table 6). A 100% susceptibility of *Candida tropicalis* and *Candida parapsilosis* to Voriconazole has been observed by some other researchers [17,16] which conflicts with the present study. Also, in a similar research carried out by [26] in Venezuela, none of the *Candida* species was found to be resistant to Voriconazole. In another study by [24] in Turkey, all 200 (100%) isolates of *Candida* species were susceptible to Voriconazole. [27] recorded 89.9% susceptibility of all the *Candida* species isolated from samples from oral candidiasis and diaper dermatitis lesions collected from children referring to private and public clinics in Ilam, Iran. This 89.9% susceptibility is much higher than the 52.3% observed in the present study. These variations in susceptibility profile may be explained by the differences in the hospital, the underlying disease of the patient, clinical specimen analyzed as well as the geographical location where the studies were carried out [26].

Table 4. *In vitro* Susceptibility Profile of the *Candida* Species to Amphotericin B (20µg)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	12(60.0)	3(15.0)	5(25.0)
Candida tropicalis	34	27(79.4)	3(8.8)	4(11.8)
Candida parapsilosis	21	16(76.2)	0(0.0)	5(23.8)
Candida krusei	7	3(42.9)	1(14.3)	3(42.9)
Candida glabrata	6	3(50.0)	3(50.0)	0(0.0)
Total	**88**	**61(69.3)**	**10(11.4)**	**17(19.3)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

Table 5. *In vitro* susceptibility profile of the *Candida* species to Flucytosine (1µg)

Species	Total number	S (%)	I (%)	R (%)
Candida albicans	20	0(0.0)	0(0.0)	20(100.0)
Candida tropicalis	34	4(11.8)	6(17.6)	24(70.6)
Candida parapsilosis	21	2(9.5)	0(0.0)	19(90.5)
Candida krusei	7	0(0.0)	0(0.0)	7(100.0)
Candida glabrata	6	0(0.0)	0(0.0)	6(100.0)
Total	**88**	**6(6.8)**	**6(6.8)**	**76(86.4)**

Key: S = Susceptible; I = Intermediate; R = Resistant

Table 6. *In Vitro* susceptibility profile of the *Candida* species to Voriconazole (1µg)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	7(35.0)	5(25.0)	8(40.0)
Candida tropicalis	34	18(52.9)	2(5.9)	14(41.2)
Candida parapsilosis	21	14(66.7)	3(14.3)	4(19.0)
Candida krusei	7	4(57.1)	0(0.0)	3(42.9)
Candida glabrata	6	3(50.0)	1(16.7)	2(33.3)
Total	**88**	**46(52.3)**	**11(12.5)**	**31(35.2)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

3.7 *In vitro* Susceptibility Profile of the *Candida* Species to Itraconazole

Twenty-seven (30.7%) of the *Candida* isolates were susceptible while 21 (23.9%) and 40 (45.5%) were susceptible dose-dependent and resistant respectively. *Candida parapsilosis* was the most susceptible (42.9%) followed by *Candida tropicalis* (35.3%) while *Candida glabrata* was the most resistant (83.3%) followed by *Candida albicans* (65.0%). There were no susceptible isolates of *Candida glabrata* (Table 7). [26] reported 27.6% resistance of *Candida* isolates in Venezuela while [27] recorded 38.3% susceptibility in Iran. In one study, 85.7% of *Candida parapsilosis* and more than 90% of *Candida tropicalis* isolates were susceptible to Itraconazole [16]. In their research, [27] found out that the resistance of *Candida albicans* to Itraconazole was 43.8% which is lower than that (65.0%) observed in the present study. There were no susceptible isolates of *Candida glabrata* which does not agree with the 83.4% susceptibility reported by [16].

3.8 *In vitro* Susceptibility Profile of the *Candida* Species to Nystatin

Table 8 shows the *in vitro* susceptibility profile of the *Candida* isolates to Nystatin. It can be seen from the table that 33 (37.5%), 16 (18.2%) and 39 (44.4%) of the isolates were susceptible, susceptible dose-dependent and resistant respectively. *Candida glabrata* was the most susceptible (66.4%) followed by *Candida parapsilosis* (52.4%) while *Candida albicans* was the most resistant (55.0%) followed by *Candida tropicalis* (50.0%) and *Candida krusei* (42.9%). This disagrees with the findings of [28] and [19] that showed a 100% susceptibility of all *Candida* isolates to Nystatin. [21] reported that out of 139 *Candida* isolates, 26 (18.7%) and 113 (81.3%) were sensitive and resistant to Nystatin respectively which does not agree with the results of the present study. [27] observed 95.3% susceptibility of all different *Candida* species in Ilam, Iran. *Candida glabrata* was the most susceptible (66.4%) followed by *Candida parapsilosis* (52.4%) while The resistance by *Candida albicans, Candida tropicalis* and *Candida krusei* against Nystatin in this study is much lower than the 70.7%, 100% and 82.0% respectively reported by [21].

In the overall, the highest susceptibility was recorded for Amphotericin B to which 61 (69.3%) of the 88 *Candida* isolates were susceptible followed by Voriconazole (52.3%) and Clotrimazole (43.0%). [27] also recorded the highest susceptibility (99.3%) by all different *Candid*a isolates to Amphotericin B. [29] detected 100% susceptibility to Amphotericin B by all strains of *Candida* isolated in Turkey. [30] reported that all of 50 *Candida* species (except 1 strain of *Candida tropicalis*) isolated in Central-Western Brazil were susceptible to Amphotericin B. The present study also agrees with that of [22] who reported that Amphotericin B was the most effective drug to which all isolates of *Candida* species except *C. krusei* were 100% sensitive. Other reports of very high susceptibility of *Candida* species to Amphotericin B have been

Table 7. *In vitro* susceptibility profile of the *Candida* species to itraconazole (50µg)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	4(20.0)	3(15.0)	13(65.0)
Candida tropicalis	34	12(35.3)	11(32.4)	11(32.4)
Candida parapsilosis	21	9(42.9)	4(19.0)	8(38.1)
Candida krusei	7	2(28.6)	2(28.6)	3(42.9)
Candida glabrata	6	0(0.0)	1(16.7)	5(83.3)
Total	**88**	**27(30.7)**	**21(23.9)**	**40(45.5)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

Table 8. *In vitro* susceptibility profile of the *Candida* species to nystatin (100units)

Species	Total number	S (%)	SDD (%)	R (%)
Candida albicans	20	6(30.0)	3(15.0)	11(55.0)
Candida tropicalis	34	9(26.5)	8(23.5)	17(50.0)
Candida parapsilosis	21	11(52.4)	3(14.3)	7(33.3)
Candida krusei	7	3(42.9)	1(14.3)	3(42.9)
Candida glabrata	6	4(66.4)	1(16.7)	1(16.7)
Total	**88**	**33(37.5)**	**16(18.2)**	**39(44.4)**

Key: S = Susceptible; SDD = Susceptible Dose Dependent; R = Resistant

documented [17,31,24]. Owing to its high toxicity (especially nephrotoxicity) and low bioavailability (when administered orally), Amphotericin B is not regularly prescribed or used extensively. This may account for the high sensitivity when compared with other antifungal drugs [22]. Amphotericin has a broad spectrum of action and presents a low incidence of fungal resistance even after a half century of clinical use. One major disadvantage of Amphotericin B is its nephrotoxicity [32,33].

The highest resistance was observed with Flucytosine to which 76 (86.4%) out of the 88 *Candida* isolates were resistant followed by Ketoconazole (61.4%) and Fluconazole (52.3%). Fluconazole has been one of the most widely used drugs for treating candidiasis [34]. In fact, Fluconazole is the most widely used drug for treating candidiasis [35] generally, and is the most commonly prescribed antifungal used for most *Candida albicans* infections [36]. Thus, wide spread and prolonged use of azoles promote rapid development of the phenomenon of multidrug resistance, which poses a major problem in antifungal therapy [34]. In the present study, Voriconazole was the second antifungal drug to which most (52.3%) of the *Candida* species were susceptible. Being a second-generation, synthetic triazole derivative of Fluconazole, it can be used to treat infections caused by Fluconazole-resistant *Candida* species [37]. Meanwhile, the highest number of susceptible-dose dependent *Candida* isolates was observed with Ketoconazole (25%), followed by Clotrimazole and Itraconazole, each recording 23.9% and then, Nystatin (18.2%). The *Candida* isolates categorized as being susceptible-dose dependent (SDD) is in recognition that yeast susceptibility is dependent on achieving maximum blood levels. Thus, an isolate with an SDD category implies clinical efficacy when higher than normal dosage of a drug can be used and maximal possible blood levels achieved [38].

Candida tropicalis was the species with the highest susceptibility (79.4%) to Amphotericin B followed by *Candida parapsilosis* (76.2%) and 66.7% respectively to Amphotericin B and Voriconazole. Fig. 1 shows the activity of some of the antifungal drugs against *Candida tropicalis*. *Candida krusei* was the species with the least susceptibility showing 0% susceptibility to each of Fluconazole, Ketoconazole and Flucytosine (Fig. 2). It is critically noted that Fluconazole is not recommended for *Candida krusei* and it has also been stated that *Candida krusei* should not be tested against Fluconazole to which it is intrinsically resistant [39]. In a review by [40], it was documented that out of 1, 075 *Candida krusei* isolates tested against Fluconazole, 96.6% was resistant to the drug. Also, *Candida glabrata* showed a 0% susceptibility to each of Flucytosine and Itraconazole while *Candida albicans* showed 0% susceptibility to Flucytosine only. *Candida glabrata* has been documented of being able to develop high-level resistance after exposure to azole antifungals [41]. Also *Candida glabrata* was the only *Candida* species with 0% resistance to Amphotericin B.

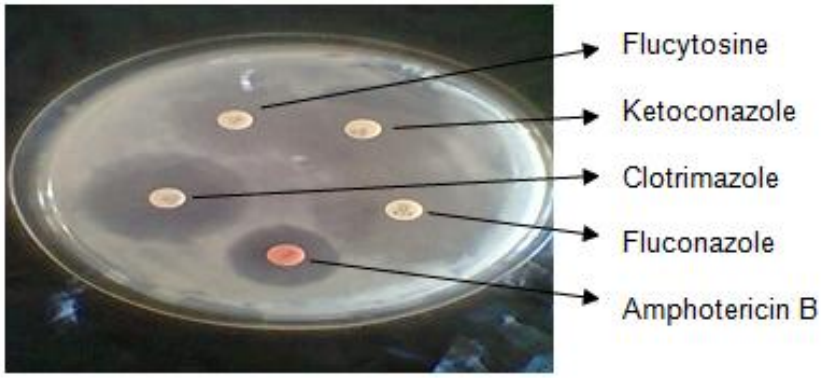

Fig. 1. Activities of some of the antifungal drugs against *Candida tropicalis*

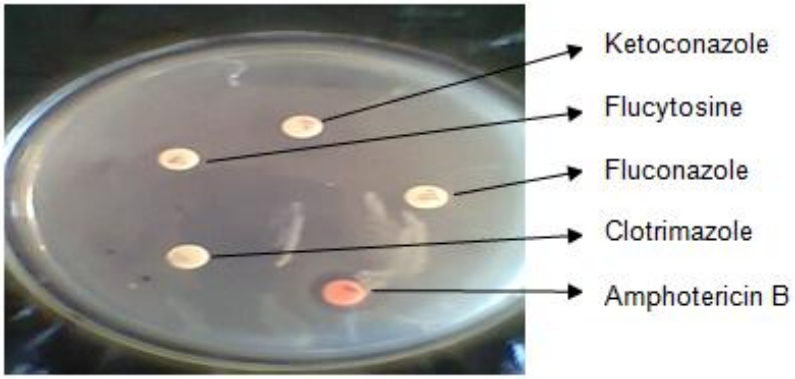

Fig 2. Total resistance against some of the antifungal drugs by *Candida krusei*

4. CONCLUSION

In the present study, the percentages of *Candida* species resistant to Fluconazole, Ketoconazole, Voriconazole, Nystatin, Amphotericin B, Flucytosine, Clotrimazole and Itraconazole were respectively 52.3, 61.9, 35.2, 44.3, 19.3, 86.4, 34.1 and 45.5%. *Candida krusei* was the most resistant species with 100% resistance to each of Fluconazole, Ketoconazole and Flucytosine. *Candida tropicalis* was the species with the highest susceptibility (79.4%) to Amphotericin B followed by *Candida parapsilosis*. The drug to which most of the *Candida* species were susceptible was Amphotericin B followed by Voriconazole while Flucytosine was the drug with the highest resistance followed by Ketoconazole and Fluconazole.

Based on the findings of the present study, Voriconazole is recommended for vaginal candidiasis especially in the study area and also especially for infections caused by Fluconazole-resistant *Candida* species. This study also recommends that sensitivity testing be carried out before antifungal therapy. Due to the fact that indiscriminate use of drugs (including antifungal drugs) is generally common in this part of the world, it should be avoided to reduce the development and spread of resistance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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