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Prevalence of Vaginal Infection by Multidrug Resistant *Candida* Species among Different Ages in Egypt

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Abstract During 12 months' the period of study from December 2015 to November 2016, one hundred and sixty women were clinically examined for vaginal yeast infection. Result revealed that 100 cases (62.5%) proved to have vaginal infection. The mean age (±SD) of the participants was 27.70±5.79 years. Most of the positive cases with high count belonged to the age group1 (19-25 year) followed by group 2 (26-30 year) representing 39 and 32 cases respectively (71 % of the total cases). Phenotypic and genotypic characterization of the yeast isolates showed that *C. albicans* were the predominant *Candida* species 258 cfu out of 410 cfu represent (62.9%) followed by *C. lusitaniae* (56 cfu, 13.7%), *C. krusei* (44 cfu 10.7%), *C. glabrata* (26 cfu, 6.3%), *C. tropicalis* (18 cfu, 4.4%) and *C. Parapsilosis* (8 cfu, 2.0%) respectively. *In-vitro* sensitivity test showed high resistance to the tested antifungals; whereas 73 isolates out of 128 (57.3%) were resistant to more than one type of tested antifungal agents, including 14 isolates showed resistance to all antifungal agents (Multidrug resistant strains). *C. albicans* represented by 67 isolates, showed resistance to one or more of the tested antifungal agents such as Fluconazole (61.2 %), Ketoconazole (56.7 %), Amphotericin B (52.2), Itraconazole and Nystatin (43.3 %). In case of non- *albicans* isolate (61 isolate) the most effective antifungal agent was Nystatin were it susceptible 65 % form *C. lusitaniae* isolates, 62.5 % from *C. tropicalis* and 60 % from *C. Krusei* isolates. The relation between risk factors, such as pregnancy, diabetes mellitus, history of antibiotic uses, and contraceptive methods, was recorded.

Keywords: *vulvovaginal candidiasis, antifungal agents, multidrug resistant, phenotypic and genotypic characterization*

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1. Introduction

Vulvovaginal candidiasis (VVC) or *Candida* vaginitis; is a common fungal infection of the vagina attributed to an overgrowth of *Candida* species. It is one of the most common medical problem throughout the world which affecting a large proportion of healthy women during their life time [1].

Candida species considered as a part of the normal microbiota of the mucous membrane of the respiratory, gastrointestinal and female genital tracts [2]. overgrowth of *Candida* in the vagina produces symptoms include vaginal and vulvar pruritus, itching, pain, sexual dysfunctions, dryness, cracks, and odorless thick, white vaginal discharge [3]. Vaginal symptoms are one of the most common reasons for consulting gynecologist for approximately 10 million visits each year [4]. In recent years, the number of vaginal yeast infections has been dramatically increasing. It is estimated that about 75% of all women infected by *Candida* spp. at least once during their reproductive years, and approximately between 40-50% during the childbearing years suffer from recurrent chronic infection [5,6]. *Candida albicans* is indicated as the most common causative pathogen of vulvovaginal candidiasis which represent about 80–95% [4,7,8,9]. However, recently there are many reports from different countries indicate that about 10-30% of the patients infected with VVC are due to non-*albicans Candida* species as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* [10,11,12,13,14]. In recent years, number of resistant vaginal yeast pathogen has been dramatically increasing, and several reports indicated differences in the distribution of *Candida* spp. associated with VVC and their antifungal drug susceptibility patterns from different geographic locations [15,16,17,18]. Mårdh *et al.* (2002) reported that up to 7.5% of vaginal *Candida* isolates have been resistant to one or more of the commonly used azoles [19]. In addition, recently non-*albicans* species have shown resistance to the antifungals commonly used to treat vulvovaginal candidiasis [20,21,22]. The appearance of resistant *candida* to certain antifungal may play a role in cases of recurrent vulvovaginal candidiasis. In a study conducted by Richter *et al*. (2005), fluconazole resistance was observed among 15.2% and 41.7% of vaginal isolates of *C. glabrata* and *C. krusei*, respectively [23]. Another study conducted by Consolaro *et al*. (2005), reported that vaginal isolates of non-*albicans* species, as *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *S. cerevisiae* were susceptible to nystatin, ketoconazole and Itraconazole [24]. There are many reasons for the emergence of resistances;

prolonged exposure and increased use of antifungals for recurrent vulvovaginitis candidiasis are the most common risk factors for resistance [20,25,26]. Furthermore, the distribution of these infections may depend on numerous risk factors that have been associated with VVC such as, pregnancy, uncontrolled diabetes mellitus, obesity, poor personal hygiene, poor dietary habits, and uses contraceptives [27,28].

This study was carried out to determine the prevalence of clinical multidrug resistant *Candida* species in different age groups of women diagnosed as having Vulvovaginal candidiasis. In addition, the possible risk factors in relation to resistance profile was also considered.

2. Materials and Methods

2.1. Patient

This study was conducted on 160 women age ranged from (19-40 years) with signs and symptoms of vulvovaginal candidiasis; who visited three private women's health centers in Assiut city, Egypt during the period from December 2015 to November 2016.

2.2. Cases of VVC

Typical symptoms of VVC includes vulvovaginal irritation, soreness, purities, change in vaginal discharge to thick white adherent vaginal discharge and growth of *Candida* spp. [29].

2.3. Sampling and Data Collection

Vaginal swabs were taken by a physician from women with a symptom of Vulvovaginal candidiasis. Clinical information was also recorded in questionnaire. this information comprised a date of sampling, age of patients, possible risk factors such as pregnancy, diabetes mellitus, contraceptive methods and history of antimicrobial uses. Samples were obtained by a sterile cotton swab, and directly placed onto 10 ml sterile normal saline (0.85% NaCl, pH 7), transported in cooler boxes to the Lab, for analysis. This study included only the patients who accepted to participate in the study.

2.4. Mycological Analyses

2.4.1. Culturing of Specimens

Under aseptic conditions, the collected specimen swabs were directly placed into 90 ml sterile normal saline (0.85% NaCl, pH 7), vortexed, then 1 ml of the suspension was streaked onto sterile Petri plates containing Sabouraud Dextrose Agar (SDA)- Difco, supplemented with chloramphenicol (250 mg/ L) -Sigma, in replicate; and incubated at 37°C, for 48-96 h. The growing colonies were counted, recorded and kept in SDA slants for further study.

2.4.2. Direct Microscopic Examination (DME)

Smears from the collected sample stained with lactophenol cotton blue (LPCB) were prepared. Appearance of budding cells with or without pseudohyphae under microscope indicates positive results [30].

2.5. Identification of Isolates

Phenotypic and genotypic characteristics were used to identify the different isolates of *Candida*.

2.5.1. Phenotypic Identification

Growth on HiCrome *Candida* Differential Agar

Is a selective and differential medium, allows differentiation of *Candida* species. According to the manufacturer *C. albicans* appears as light green colored smooth colonies, *C. tropicalis* blue to metallic blue, *C. glabrata* cream to white smooth colonies, *C. krusei* purple fuzzy colonies and *C. lusitaniae* pale to pink colonies [31].

2.5.2. API *Candida* (BioMérieux).

A commercial Analytical Profile Index (API) yeast identification kit, namely API *Candida* (bioMérieux) was used to conduct the biochemical tests on the isolated *Candida* spp. The API *Candida* strip consists of 12 biochemical tests. Five carbohydrate-acidification tests (glucose, galactose, saccharose, trehalose, and raffinose) and seven enzymatic tests (*β*-maltosidase, *α*-amylase, *β*-xylosidase, *β*-glucuronidase, urease, Nacetyl-*β*-glucosaminidase, and *β*-galactosidase). Inoculum yeast suspension was prepared, inoculated to the strip and incubated for (24 hours at a temperature of 35°C), visual color reaction was observed. The results were compared with those given in the profile list in the package insert. One of the strips was used as negative control where sterile distilled water was used as inoculum.

2.5.3. Genotypic Identification

Some isolates which have revealed high resistance to the tested antifungal agents were selected. Nucleotide sequencing of rRNA gene was done at Solgent Company, Daejeon, South Korea. Primers used for gene amplification have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then the amplification was carried out in a thermal cycler under the following conditions: one round of denaturation at 95°C for 15 sec followed by 30 cycles of denaturation at 95°C for 20 sec, annealing at 50°C for 40 sec and extension at 72°C for 1 min, with a final extension step at 72 °C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. Purified PCR products were reconfirmed (using size marker) by electrophoreses on 1% agarose gel. Then the bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Each sample was sequenced in the sense and antisense directions using ITS1 and ITS4 primers [32]. Sequences were further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of software DNA Star version 14.

2.6. Antifungal Susceptibility Testing

A total of 128 isolate of *Candida* species obtained from patients diagnosed with VVC were tested for their susceptibility to eight standard antifungal agents namely; Fluconazole (10 μg), Amphotericin B (100 units), Nystatin (100 units), Ketoconazole (10 μg), Clotrimazole (10 μg), Itraconazole (10 μg), Miconazole (30 μg) and Voriconazole (1 μg) (Himedia, India) by disk diffusion method according to the procedure described in the Clinical and Laboratory Standards Institute (CLSI) [33]. Briefly, each isolate was sub-cultured on SDA and an inoculum was suspended in sterile normal saline and the turbidity was adjusted to 0.5 McFarland standard and streaked on the surface of sterilized Mueller-Hinton agar (MHA) (Oxoid). The antifungal agent discs were placed onto seeded agar plates and incubated at 37°C, for 24 h. The diameter of inhibition zone was measured and recorded. The antifungal agent, concentration, abbreviation and interpretative breakpoints are shown in Table 1.

3. Results and Discussion

3.1. Patient Age in relation to VVC

During the period study (12 months), a total of 160 samples were collected from different age groups with signs and symptoms of vulvovaginal candidiasis. The direct microscopic examination; revealed that only 59 out of 160 cases (36.9 %) were positive showing budding yeast cells and/or pseudohyphae. However, the number of positive culture on SDA was higher (100 patients representing 62.5 % of total samples). The mean age of the studied patient was 27.70±5.79 and the average total count of yeast colony isolated were 410 cfu for all samples. 78 % from the culture positive cases showed a single yeast growth whereas other 22 % showed mixed growth**.** Most of the positive cases with high count belonged to the age group 1 (19-25 year) and group 2 (26-30 year) given 39 and 32 cases respectively representing 71 % of total cases as shown in Table 2 & Figure 1. This result is slightly more than those obtained by Nurat *et al*. (2015) who reported that, the prevalence of VVC was 25% and the highest numbers of VVC were in age group 21-25 years represent (40.44%) followed by 26-30 years with (32.58%) [34]. Benchellal *et al.* (2011) reported that the commonest age group to have positive VVC was from 26-30 [35]. Holland *et al.* (2003) reported that the mean age of patients with *C. albicans* and non-*albicans* species was 33±13.5 and 43±15.9 years old, respectively [36]. Kent (1991) reported that, the women who are younger and sexually active are more vulnerable to the infected with *Candida* species [37].

3.2. Risk factors

The risk factors, including pregnancy, diabetes mellitus, uses of contraceptives, and history of antimicrobial uses were assessed in all positive cases (Table 3). Out of all cases, 82 % of patients were non-pregnant status.

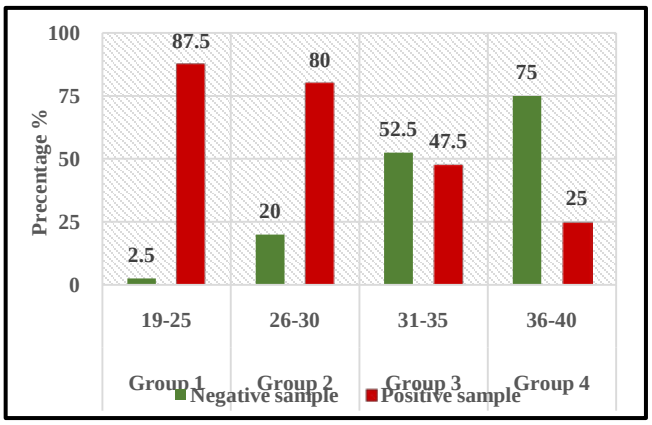

Figure 1. Incidence of vaginal yeast infection in relation to age group

Table 1. Antifungal agent, Abbreviation, Potency and Interpretative breakpoints

Antifungal agents	Abbr.	Potency	Inhibition Zone diameter (mm)		
			Susceptible	Intermediate	Resistant
Amphotericin B	AP	100 units	≥15	14-11	≤ 10
Clotrimazole	CC	10 µg	≥20	19-12	≤ 11
Fluconazole	FLC	10 µg	≥19	18-15	≤ 14
Itraconazole	IT	10 µg	≥23	22-14	≤ 13
Ketoconazole	KT	10 µg	≥28	27-21	≤ 20
Miconazole	MIC	30 µg	≥20	19-12	≤ 11
Nystatin	NS	100 units	≥15	14-11	≤ 10
Voriconazole	VOC	1 µg	≥17	16-14	≤ 13

Table 2. Total number of the collected sample (positive and negative) classified according to age group

Age group (year)		**Total Sample (160)**	**Negative sample**		**Positive sample 100 cases (62.5%)**					
					Albicans		**Non- *albicans* (NAC)**		**Total**	
			N	%	N	%	N	%	**N**	%
Group 1	19-25	40	1	2.5	24	61.5	15	38.5	**39**	**87.5**
Group 2	26-30	40	8	20.0	21	65.6	11	34.4	**32**	**80.0**
Group 3	31-35	40	21	52.5	8	42.1	11	57.9	**19**	**47.5**
Group 4	36-40	40	30	75.0	6	60.0	4	40.0	**10**	**25.0**
Total		**160**	**60 (37.5)**		**59**		**41**		**100**	**62.5**

Table 3. Distribution of risk factors among positive cases of patients with vulvovaginal candidiasis infection

Age group	Group 1 (39 cases)		Group 2 (32 cases)		Group 3 (19 cases)		Group 4 (10 cases)		Total positive (100 cases)
	Albicans	NAC	*Albicans*	NAC	*Albicans*	NAC	*Albicans*	NAC	
1- Pregnancy									
Pregnant	5	2	3	4	2	0	1	1	**18**
Non-pregnant	19	13	18	7	10	7	3	5	**82**
2- Diabetes mellitus									
	2	0	0	0	0	0	4	1	**7**
3- Contraceptive									
None	18	9	6	3	3	0	0	0	**39**
Oral	0	0	0	0	5	1	2	2	**10**
Device	8	4	14	9	7	3	6	0	**51**
4- Antimicrobial uses									
None	11	15	2	2	9	5	4	2	**50**
Antibiotic	8	2	15	4	1	2	1	3	**36**
Antifungal	2	1	2	7	1	1	0	0	**14**
5- previous infection VVC Since 2 months later									
	13		8		4		0		**25**

Only 7 cases have diabetes mellitus. About 39 % of patients doesn't use any contraception tools whereas others use either oral contraceptive (10 cases) or an intrauterine contraceptive device (51 cases). In a total, 36 % of the patients had a history of antibiotic therapy, and 14 % using of antifungal during or two months before sample collection. A history of previous VVC episodes was reported by 25 patients all of them had previous history of using antimicrobial agents. In the respect, Kanagal *et al.* (2014) highlights that there are several factors associated with increased rates of *Candida* vaginitis including diabetes, previous candidiasis infection, use of antibiotics and different forms of contraceptive [38]. Nelson *et al.* (2013) reported also that in pregnant women, vaginal candidiasis has been related to emotional stress and suppression of immune system. This high prevalence of vaginal candidiasis may lead to pregnancy complications like abortions, premature birth, low birth weight and other morbidities. [39,40]. Another main factor of prevalence of VVC include the misuse of antibiotics for treatment of such infections. This misuse of drugs results in resistance especially to the common antifungal agents.

3.3. Strain Identification and Distribution

3.3.1. Phenotypic Identification

In the current study, six species of *Candida* were identified; *C. albicans* were the predominant *Candida* species (258 cfu out of 410 representing 62.9%) followed by *C. lusitaniae* (56 cfu, 13.7%), *C. krusei* (44 cfu, 10.7%). *C. glabrata* (26 cfu, 6.3%), *C. tropicalis* (18 cfu, 4.4%) and *C. Parapsilosis* (8 cfu, 2.0%) respectively. *Rhodotorula sp.* was represented with (23 cfu, 5.6 %) Table 4 and Figure 2, Figure 4.

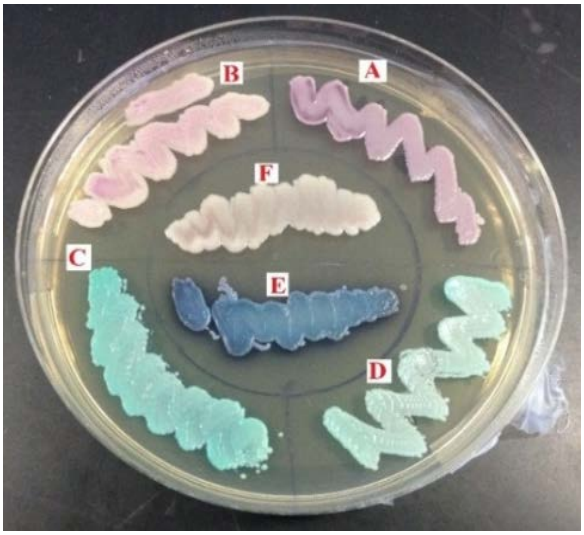

Figure 2. HiCrome agar plate showing different colors of *Candida* species. (A) purple fuzzy colonies for *Pichia kudriavzevii* (anamorph *Candida krusei*) (B) Light pink for *Clavispora lusitaniae*, (teleomorph of *Candida lusitaniae*) (C) dark green colored for *C. Parapsilosis*, (D) light green colored smooth colonies, for *C. albicans*, (E) blue to metallic blue colored raised colonies for *C. tropicalis* (F) cream to white smooth colonies for *C. glabrata*

3.3.2. API *Candida* (BioMérieux).

A group of API *Candida* strips were employed to confirm the identification of yeast isolates as shown in Table 5.

3.3.3. Genotypic identification

Molecular characterization rRNA gene sequencing and establishment of the phylogenetic tree confirmed the phenotypic identification of *Candida* species Figure 3.

Table 4. Incidence of the different isolated *Candida* species in relation to age group

Species	***Albicans* (258 isolate) 62.9 %**			**Non- *albicans* (152 isolate) 37.1 %**									
Age	No of cases	*C. albicans*		*C. lusitaniae*		*C. Krusei*		*C. glabrata*		*C. tropicalis*		*C. Parapsilosis*	
		N	**%**	N	**%**	N	**%**	N	**%**	N	**%**	N	**%**
Group 1	**39**	113	**27.6**	22	**5.4**	18	**4.4**	9	**2.3**	10	**2.4**	6	**1.5**
Group 2	**32**	79	**19.3**	16	**36.4**	21	**5.1**	15	**36.6**	5	**1.2**	2	**0.5**
Group 3	**19**	49	**12.0**	10	**2.4**	4	**1.0**	1	**0.2**	3	**0.7**	0	**0.0**
Group 4	**10**	17	**4.1**	8	**2.0**	1	**0.2**	1	**0.2**	0	**0.0**	0	**0.0**
Total	100	**258**	**62.9**	**56**	**13.7**	**44**	**10.7**	**26**	**6.3**	**18**	**4.4**	**8**	**2.0**

Table 5. API *candida* identification kits, used to conduct the biochemical test

Isolate	*C. albicans*	*C. lusitaniae*	*C. krusei*	*C. glabrata*	*C. tropicalis*	*C. Parapsilosis*
Code	7002	7500	1000	1100	7530	7310
GLU	+	+	+	+	+	+
GAL	+	+	-	-	+	+
SAC	+	+	-	-	+	+
TRE	-	+	-	+	+	+
RAF	-	-	-	-	-	-
β-MAL	-	+	-	-	+	+
α- AMY	-	-	-	-	+	-
β-XYL	-	-	-	-	+	-
β-GUR	-	-	-	-	-	-
URE	-	-	-	-	-	-
β-NAG	+	-	-	-	-	-
β-GAL	-	-	-	-	-	-

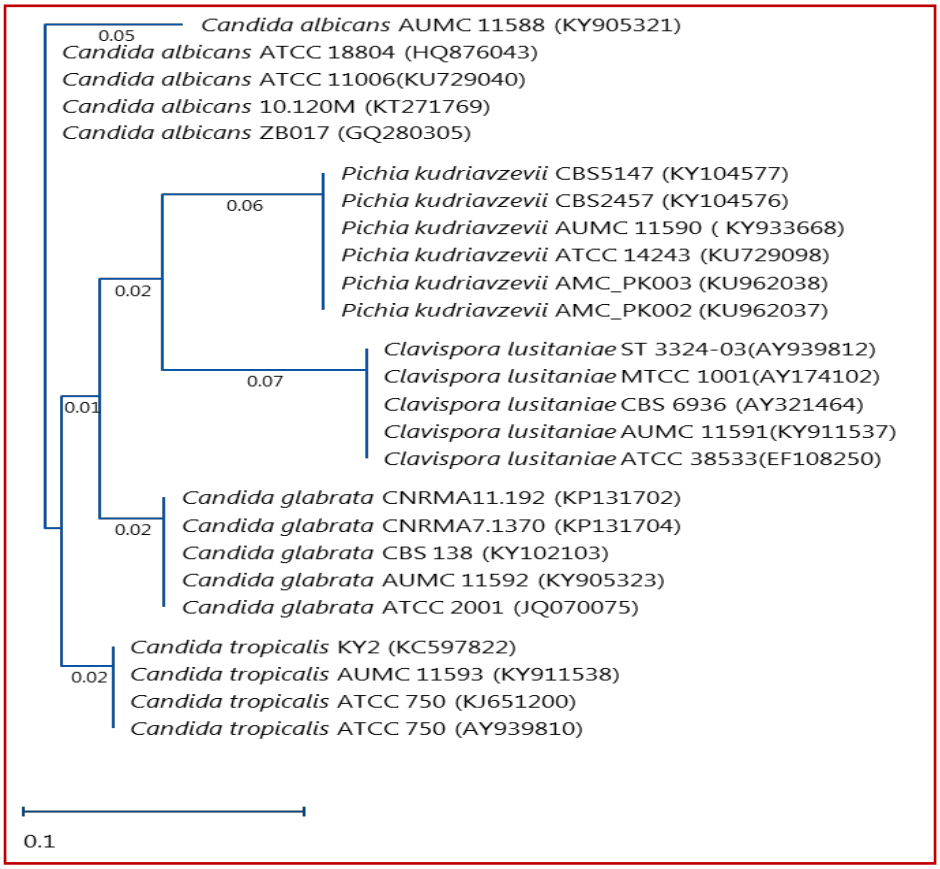

Figure 3. Phylogenetic relationship of the multidrug resistant *Candida* isolates recovered from vulvovaginal candidiasis and closely related species based on 18S rRNA gene sequencing. Each isolate given AUMC No. and the GenBank accession number in parentheses

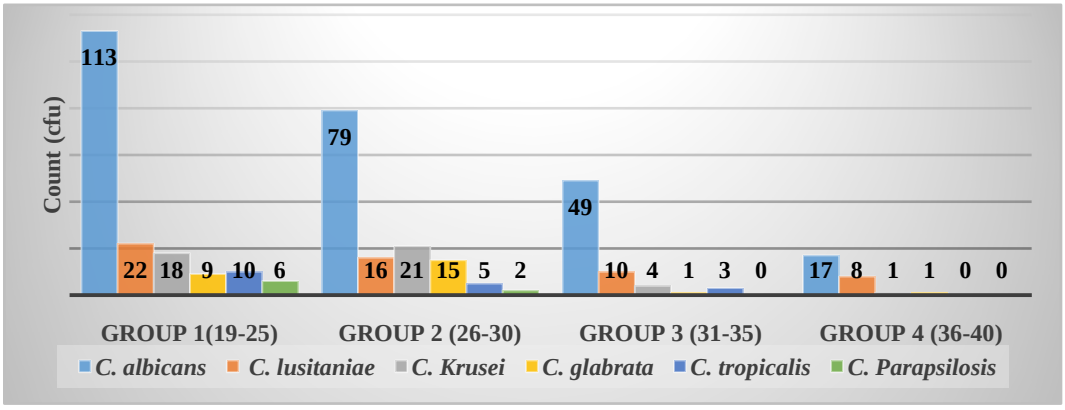

Figure 4. Incidence of the different isolated *Candida* species in relation to age group

Many investigations from different countries had shown that *C. albicans* was the most common species identified in patients with VVC; varying from 46.9% to 76.3% [41,42,43,44]. In the same context, the present study highlights that *C. albicans* (62.9 %) is still the predominant pathogen causing VVC. and the remaining 37.1% were other non-*albicans* species. The distribution of *Candida* species varies greatly depending on the geographical areas as well as the population habits. Recently, there has been an increasing trend in the isolation rate of non-*albicans* species reported from some countries, such as *C. glabrata* was the most common species isolated from the vaginal swabs with isolation rate ranged from 30 to 71 % from non- *albicans* isolates [45,46,47,48,49]. In contrast to most studies, our study revealed that the non- *albicans*, *C. lusitaniae* is the second most common species, and *C. krusei* disbanded in third place. According to Nyirjesy*et al.* (1995) an increase of non-*albicans* species has been observed, particularly in recurrent cases [50].

3.4. Sensitivity of Yeasts to Antifungal Agents

In the present study, 8 different standard antifungal agents were evaluated against 128 isolates of *candida* species (*C. albicans* 67 isolates, *C. lusitaniae* 23 isolates, *C. krusei* 20 isolates, *C glabrata* 8, *C. tropicalis* 2, and *C. Parapsilosis* 2). The results showed a high frequency of resistant isolates to the tested antifungals; It was found that only 4 isolates (3.1%) sensitive to all antifungal agents, 51 isolates (39.8%) showed intermediate effect, whereas 73 isolates (57.3%) showed resistant to more than one type of antifungal agent, including 14 isolates showed resistance to all tested antifungal agents (Multidrug resistant strain). *Candida* from the age group 1 (19-25) year (24 out of 39 cases, 61.5%) were the most resistant. Followed by the second highest group 2 (31-35) with 16 out of 32 cases (50.0 %) Table 7 and Figure 5.

Results in the Table 7 revealed that *C. albicans* (represented by 67 isolates) showed resistance to one or more of the frequently prescribed antifungal agents such as Fluconazole (61.2 %), Ketoconazole (56.7 %), Amphotericin B (52.2%), Itraconazole and Nystatin (43.3 %). In case of non- *albicans* isolates (61 isolate) the most effective antifungal agent was Nystatin, it susceptible 65 % of *C. lusitaniae* isolates, 62.5 % of *C. tropicalis* and 60 % of *C. Krusei* isolates.

Many studies have found that the resistance rates of *C. albicans* are 8.6% for voriconazole, 19.1% for Itraconazole, 9.7% for fluconazole, 2.6% for ketoconazole and 12.7% for miconazole [17,18]. In 2002, a study from Belgium reported an alarmingly high fluconazole resistance rate of 21% in 84 vaginal *C. albicans* isolates [12]. An earlier study reported fluconazole non-susceptibility in 67% of vaginal *Candida* isolates [23]. Liu *et al* (2014) showed that the resistance rates to *C. albicans* were 1.1, 2.2 and 4.2% for fluconazole, Itraconazole and miconazole, respectively [51]. *Candida* infection treatments, which fail to respond to conventional antifungal drug treatments, have become increasingly reported. Recently there are many infected cases don't respond to treatment using azole class anymore, such as Fluconazole. This is due to the widespread, long-term use of antifungal for treating and preventing yeast infection.

In conclusion, the current results indicate that *C. albicans* was the predominant *Candida* species isolated from cases of VVC, while non-*albicans* species came second. The high frequency of the resistant strains found in our study give an alarming to demonstrate further studies to evaluate *in vitro* susceptibility of clinical yeast isolates. Successful treatment of vaginal yeast infections can be helpful in precise diagnosis of VVC and control the prevalence of resistant *candida*.

Table 6. Resistant prevalence for positive sample

Age group (year)		Total No of cases (cfu)	Susceptibility profile			
			Sensitive 4 cases (cfu)	Intermediate 40 cases (cfu)	Resistant (56 cases) (73 cfu)	
					More than (3 types) 45 cases	Resistant to all (8 types) 11 cases
Group 1	19-25	**39 (62)**	2 (2)	13 (26)	16 (24)	8 (10)
Group 2	26-30	**32 (35)**	2 (2)	14 (14)	15 (17)	1 (2)
Group 3	31-35	**19 (21)**	0 (0)	6 (6)	12 (14)	1 (1)
Group 4	36-40	**10 (10)**	0 (0)	5 (5)	4 (4)	1 (1)
Total		**100 (128)**	**4 cfu**	**51 cfu**	**59 cfu**	**14 cfu**

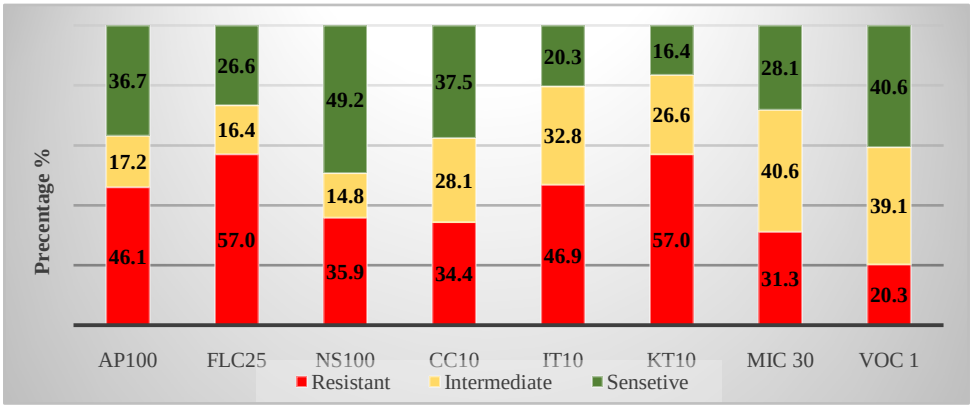

Figure 5. Susceptibility profile of 8 antifungal agents

Table 7. In vitro susceptibility profile of *Candida spp.* isolates against 8 standard antifungal agents

Antifungal agents		*Albicans* (67 isolate) 52.3 %		Non- *albicans* (61 isolate) 47.7%											
	DS	*C. albicans*		*C. lusitaniae*		*C. Krusei*		*C. glabrata*		*C. Tropicalis*		*C. Parapsilosis*		Total	
		N= 67		N= 23		N= 20		N= 8		N= 8		N= 2		128	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%
AP	R	35	52.2	6	26.1	8	40.0	4	50.0	6	75.0	0	0.0	59	46.1
	I	11	16.4	5	21.7	3	15.0	2	25.0	0	0.0	1	50.0	22	17.2
	S	21	31.3	12	52.2	9	45.0	2	25.0	2	25.0	1	50.0	47	36.7
FLC	R	41	61.2	15	65.2	9	45.0	4	50.0	3	37.5	1	50.0	73	57.0
	I	8	11.9	3	13.0	4	20.0	3	37.5	2	25.0	1	50.0	21	16.4
	S	18	26.9	5	21.7	7	35.0	1	12.5	3	37.5	0	0.0	34	26.6
NS	R	29	43.3	5	21.7	4	20.0	4	50.0	2	25.0	2	100.0	46	35.9
	I	8	11.9	3	13.0	4	20.0	3	37.5	1	12.5	0	0.0	19	14.8
	S	30	44.8	15	65.2	12	60.0	1	12.5	5	62.5	0	0.0	63	49.2
CC	R	19	28.4	7	30.4	9	45.0	3	37.5	5	62.5	1	50.0	44	34.4
	I	17	25.4	6	26.1	7	35.0	2	25.0	3	37.5	1	50.0	36	28.1
	S	31	47.0	10	15.2	4	6.1	3	4.5	0	0.0	0	0.0	48	37.5
IT	R	29	43.3	11	47.8	9	45.0	5	62.5	6	75.0	0	0.0	60	46.9
	I	19	28.4	9	39.1	8	40.0	3	37.5	1	12.5	2	100.0	42	32.8
	S	19	28.4	3	13.0	3	15.0	0	0.0	1	12.5	0	0.0	26	20.3
KT	R	38	56.7	12	52.2	9	45.0	6	75.0	6	75.0	2	100.0	73	57.0
	I	18	26.9	5	21.7	7	35.0	2	25.0	2	25.0	0	0.0	34	26.6
	S	11	16.4	6	26.1	4	20.0	0	0.0	0	0.0	0	0.0	21	16.4
MIC	R	23	34.3	6	26.1	7	35.0	3	37.5	1	12.5	0	0.0	40	31.3
	I	26	38.8	9	39.1	6	30.0	5	62.5	4	50.0	2	100.0	52	40.6
	S	18	26.9	8	34.8	7	35.0	0	0.0	3	37.5	0	0.0	36	28.1
VOC	R	16	23.9	4	17.4	4	20.0	1	12.5	1	12.5	0	0.0	26	20.3
	I	31	46.3	6	26.1	8	40.0	2	25.0	3	37.5	0	0.0	50	39.1
	S	20	29.9	13	56.5	8	40.0	5	62.5	4	50.0	2	100.0	52	40.6

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