

Prevalence and Sensitivity Patterns of Candidal Infections in Various Tertiary Care Health Subunits of Karachi

Rabeea Rizwan¹, Zahida Memon¹, Shehla Shaheen¹, Faisal Afridi², Mubarak Zaib²

¹Department of Pharmacology, Ziauddin Medical College, Karachi, Pakistan

²Department of Microbiology, Ziauddin University Hospital, Karachi, Pakistan

Email: rabeea_rizwan@hotmail.com, drshel2011@gmail.com

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Abstract

Mostly candida resides as an opportunistic organism on epithelial surfaces of human being. However, under auspicious conditions can cause infections including serious life threatening invasive candidiasis with subsequent mortality particularly in immune deficit and hospitalized patients having co-morbidities. Limited data are published on the prevalence of candidiasis, based on the researches conducted at few tertiary care settings which are not representing the overall disease burden in our country, Pakistan. Therefore, this study was conducted to evaluate the frequency and sensitivity patterns of candidiasis in our community. **Methods:** Out of total 1020 specimens, 130 clinical samples were identified as candidal positive, obtained from March to May 2018. These samples were isolated from vagina, oropharynx, urine, tracheal aspirates, pus, blood, tips of the intubations, wounds and fluids of the body cavities. Identification of candida, its species and antifungal sensitivity screening was done by Kirby Bauer's disk diffusion method according to CLSI guide lines' (M - 44 A2 series, 2009). **Results:** A significant majority, 80 (61.5%) of candidal strains were isolated from females with female to male ratio 8:5 and most of these isolates were obtained from high vaginal swabs (43.75%). Four candidal species (*Candida albicans* 80%, *Candida tropicalis* 10%, *Candida glabrata* 9.2% and *Candida ciferrii* 0.8%) were isolated from all positive specimens. Maximum number of the positive samples 52 (40%) were obtained from ICU patients. Sensitivity test of candidal positive samples revealed that commonly used azole antifungal drugs, fluconazole and voriconazole were highly resistant, with respective 57.7% and 70.8% resistance. **Conclusion:** Candidiasis is highly prevalent in our clinical set up and more frequently infecting females in comparison to males as most of the positive isolates were retrieved from HVS (high vaginal swabs). Still, *C. albicans* was found to be the most preva-

lent specie isolated among all candida samples. Our study also demonstrated that the resistance of most commonly prescribed antifungals, azoles have shown a rapid rise. Therefore, it is recommended that before prescription of antifungal drugs the clinicians should routinely recommend culture and sensitivity testing of samples taken from candida infected individuals.

Keywords

Candida, Candidiasis, Antifungal Drugs, Fluconazole, Voriconazole, *C. albicans*, Candidal Infection, Sensitivity, Resistance

1. Introduction

In daily life, human beings are commonly exposed to numerous microorganisms including fungi, among which some behave as normal commensals while other could be pathogenic. According to CDC (Centers for Disease Control), three wide classes of fungi are of concern, these are; 1) opportunistic, 2) community acquired 3) hospital acquired [1]. Candida, an ascomycetous yeast is one of the common fungi responsible for infecting individuals across the globe [2]. It is one of the normal floras of our body surfaces, acts as opportunistic organism which dwells in 70% of human population. Under certain suitable and privileged circumstances candida can infect through its resident areas like oropharynx, vagina, vulva, urethra, ears externa and nails. It can also invade the affected distorted epithelial surfaces by forming hyphae and can become the source of systemic infection. It usually causes recurrent infections in spite of prolonged antifungal therapy [3] [4] [5] [6].

Global warming is continuously rising that declines the temperature gradient between human and fungi which becomes one of the reasons of rise in fungal infections around the world [7]. Pakistan is a land of varied geographical features with extremes of seasonal variations seen across the country [8]. These geographical factors, such as hot and humid weather, heavy rain falls during monsoons and floods are thought to increase the risk of acquiring fungal infections among our population. It is usually associated with people of extreme ages, weakened or altered immunity, prolonged hospitalization and organ transplantation. Prolonged catheterization, use of chemotherapeutics, immunosuppressants, corticosteroids (topical/oral), and chronically ill individuals on polypharmacy are also associated risk factors for candidiasis. Since 19th century, the incidence and prevalence rate of mycotic diseases is continuously rising in aforementioned high-risk population [9]. Candidiasis is now becoming a grave health related issue due to continuous rise of its high morbidity and mortality rates with passing time [9].

Routinely prescribed antifungal drugs for treating candidiasis included azoles, polyenes and echinocandins. Topographic variance has been observed in the

occurrence rate and antifungal sensitivity of various candidal species as evident from the data of different countries and clinical setups around the world [10]. Therefore, this study was conducted at four subunits of a tertiary care hospital located in different areas of Karachi to evaluate the prevalence of candidiasis along with species identification and their antifungal resistance patterns in our community.

2. Materials and Methods

This prospective quasi experimental study was conducted at four subunits of a tertiary care, Ziauddin hospital located in different areas of Karachi, from March, 2018 to May, 2018. After approval from all competent committees including Ethical Review Committee of Ziauddin University, research work was initiated. After informed consent patients with signs and symptoms of fungal infections like white patchy and itchy skin or mucosal regions, additionally patients with their prolonged hospitalization and on prolonged antibiotics therapy presented with constant fatigue, recurrent infections of different regions of the body and whose laboratory test found candida positive, were included in this study. While those patients, didn't want to participate were excluded from the study. Their samples were taken from oropharynx (oral and throat swabs), blood, respiratory tract (sputum, tracheal secretions, nasopharyngeal aspirates, bronchoalveolar lavage), genitals (high vaginal swab), pus and urine for culture and sensitivity (C/S). All samples were processed in microbiology laboratory within 24 hours according to standard protocols. Patients' data was entered in electronic hospital software (LIS—Laboratory Information System).

2.1. Methodology

According to the standard procedures, yeast were identified by; BACTEC 9240™ for blood specimens, other specimens like vaginal swabs were gram stained and wet mounted. Germ tube test was also performed to identify hyphal yeast. Sabouraud dextrose agar media (SDA, Oxoid, UK) was prepared according to manufacturer's directions along with adding Chloramphenicol to inhibit bacterial growth on it. Candida was grown by streaking on media (SDA) as shown in **Picture 1**. After 48 hours of positive fungal growth, species identification was done on differential agar media, CHROME agar candida (Oxoid, UK) by incubating at 35°C for 2 days which showed different colored grown colonies of various candidal species (**Picture 2**). Species identification was further confirmed by API 20 C AUX method. At 0.5 MacFarland's, turbidity culture sensitivity test was done by 'Kirby Bauer's method and CLSI guide lines' (M - 44 A2 series, 2009) [11]. For this purpose, Muller Hinton Agar Media (MHA) was prepared according to company instructions (Oxoid, UK), along with adding 2% glucose and 0.5% Methylene blue in it. Antifungal discs of fluconazole 10 mcg and voriconazole 1 mcg (HiMedia, India) were placed on these media. After two days of incubation, zones of inhibition (ZIH) around each disc were noted to estimate the sensitivity patterns of respective antifungal drugs [12] [13].



Picture 1. Candidal positive, white velvety colonies of a specimen grown on Sabouraud dextrose agar media.



Picture 2. Green colored colonies showing *Candida albicans* positive samples, grown on differential CHROMagar media.

2.2. Data Analysis

The sample size was calculated by Sealed Envelope calculator version 2012.

The calculated sample size was 64 but for this research 100 samples by consecutive sampling technique were taken according to the inclusion and exclusion criteria.

All collected data was entered and analyzed by using SPSS (Statistical Package for the Social Sciences) version 20 and transferred to Microsoft word 2016. Numerical data (zones of inhibition) was expressed by mean and standard deviations, while categorical data (sources of isolates, age groups, gender, clinical settings, species, antifungal sensitivity and resistance patterns of isolates) were stated in frequency and percentages and their associations were tested by applying Chi-square test. P-value < 0.05 considered significant.

3. Results

3.1. Demography and Sensitivity Pattern of Candidiasis

3.1.1. Number of Samples and Distribution of Candidiasis within Different Age Groups and Gender

From 1st March 2018 to 30th May 2018, 130 (12.75%) samples were found to be candida positive out of the total 1020. The age groups of patients reported in this study with candidal infections ranged from 2 months to 89 year with mean age of 51 ± 20.27 year. The most candidal infected age group was 51 to 60 year with an overall prevalence of 26.20% (**Figure 1**). Among males the most frequently candidal infected age group reported was ranged between 51 - 60 year of age whereas among females from 31 - 40 year with highly significant p value < 0.01 (**Table 1**).

3.1.2. Distribution of Clinical Settings and Basic Demography of Candidal Patients

As shown in **Table 2**, majority (40%) of the candida positive samples were isolated from ICU patients followed by patients admitted in wards and private rooms (36.2%). It was observed that females were predominantly 80 (61.54%) infected with candidiasis in comparison to the males (**Figure 2**). In females candida positive samples were frequently associated with candidal vaginitis 35 (43.75%) in comparison to males whose candidal positive samples were mostly related to candidal infected tracheal aspirates 14 (28%) with highly significant P value (<0.01). Overall in both gender, candidiasis was frequently retrieved from vagina (26.9%) sputum (19.23%) and urine (17.7%). Candidal specimens in both genders are displayed in **Table 3**.

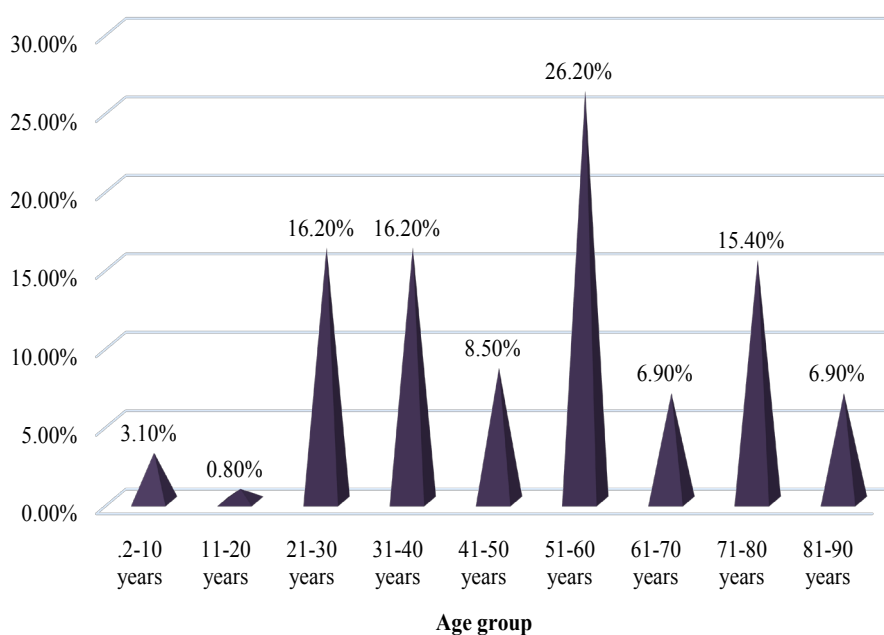


Figure 1. Frequency of candidal infections among various age groups.

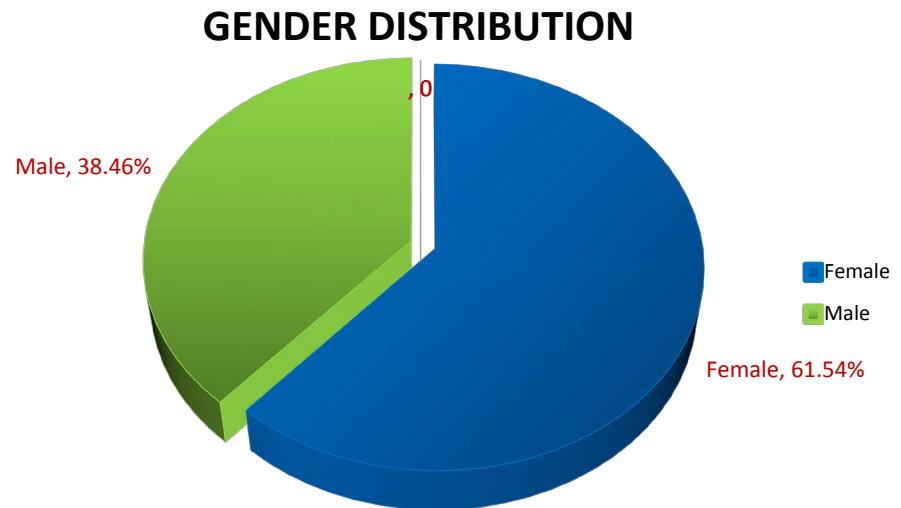


Figure 2. Frequency of gender distribution in isolated cases of candidiasis.

Table 1. Association of candidal patients' age group with their gender.

Age group	Gender		Total no. of gender in age groups	P - value
	Male 50 (38.48%)	Female 80 (61.5%)		
	n (%)	n (%)	n (%)	
0.2 - 10 years	3 (8)	0 (0)	3 (3.1)	< 0.01
11 - 20 years	1 (2)	0 (0)	1 (0.8)	
21 - 30 years	3 (6)	18 (22.5)	21 (16.2)	
31 - 40 years	2 (4)	19 (23.8)	21 (16.2)	
41 - 50 years	0 (0)	11 (13.8)	11 (8.5)	
51 - 60 years	16 (32)	18 (22.5)	34 (26.2)	
61 - 70 years	4 (8)	5 (6.2)	9 (6.9)	
71 - 80 years	14 (28)	6 (7.5)	20 (15.4)	
81 - 90 years	6 (12)	3 (3.8)	9 (6.9)	
Total	50 (100)	80 (100)	130 (100)	

Table 2. Overall frequency of number of candidal cases from different settings of Ziauddin group of hospitals in different regions of Karachi.

Clinical settings	Frequency (n)	Percent (%)	Total (%)
OPD	31	23.8	23.8
IPD - ICU/CCU/E.R/O.T	52	40.0	76.2
IPD - WARD/ADMIT/PVT ROOM	47	36.2	
Total	130	100	100

OPD—out patient department, IPD—in patient department, ICU—intensive care unit, CCU—critical care unit, ER—emergency department, OT—operation theatre, PVT ROOM—private room.

Table 3. Association of Gender with isolated candidal sources by Pearson Chi-Square.

Source of isolates	Gender				P - value
	Male (38.46%)		Female (61.54%)		
	n	%	n	%	
Ascitic fluid 1 (0.8%)	1	2	0	0	< 0.01
Blood 8 (6.2%)	5	10	3	3.75	
HVS* 35 (26.9%)	0	0	35	43.75	
N/G tube** 1 (0.8%)	0	0	1	1.25	
Peritoneal fluid 1 (0.8%)	1	2	0	0	
Pus 9 (6.9%)	9	18	0	0	
Sputum 25 (19.23%)	11	22	14	17.5	
Tip 6 (4.6%)	0	0	6	7.5	
Tracheal aspirate 17 (13.1%)	14	28	3	3.75	
Urine 23 (17.7%)	9	18	14	17.5	
Wound swab 4 (3.1%)	0	0	4	5	
Total 130 (100%)	50	100	80	100	

*HVS: High vaginal swab, **N/G: Nasogastric tube.

3.1.3. Frequency of Candidal Species

Among all candidal species, *C. albicans* was the most frequent specie isolated from 80% of the candida positive samples while among non albicans the isolated species were *C. tropicalis* 10%, *C. glabrata* 9.23% and *C. ciferrii* 0.8%. Overall 25.40% isolates showed co-infection with bacteria, as shown in **Figure 3**.

3.1.4. Sensitivity and Resistance Patterns of Clinical Isolates

Table 4 demonstrates the sensitivity and resistance patterns of identified candidal isolates against fluconazole and voriconazole. According to our results voriconazole was found to be more resistant (70.8%) in comparison to fluconazole showing resistance of 57.7% with overall resistance to both azoles was 64.25% (35.75% sensitivity). Though the cross resistance detected was (66) 50.7%.

3.2. Candidemia—A Life Threatening Invasive Blood Stream Infection

In present study out of 130 candidal positive isolates 8 specimens were obtained from blood as source of infection at a rate of 6.15%. Candidemia was observed to be 62.5% common in males in comparison to females (**Table 5**). Among species *C. non albicans* were the major offender for this blood stream infection involving *C. tropicalis* 87.5% and *C. ciferrii* 12.5% (**Table 6**). While mortality rate analyzed was 50% (**Table 5**). Among species 87.5 % of samples showed *C. tropicalis* predominance while overall sensitivity profile against both azoles showed 75% resistance, displayed in **Table 7**.

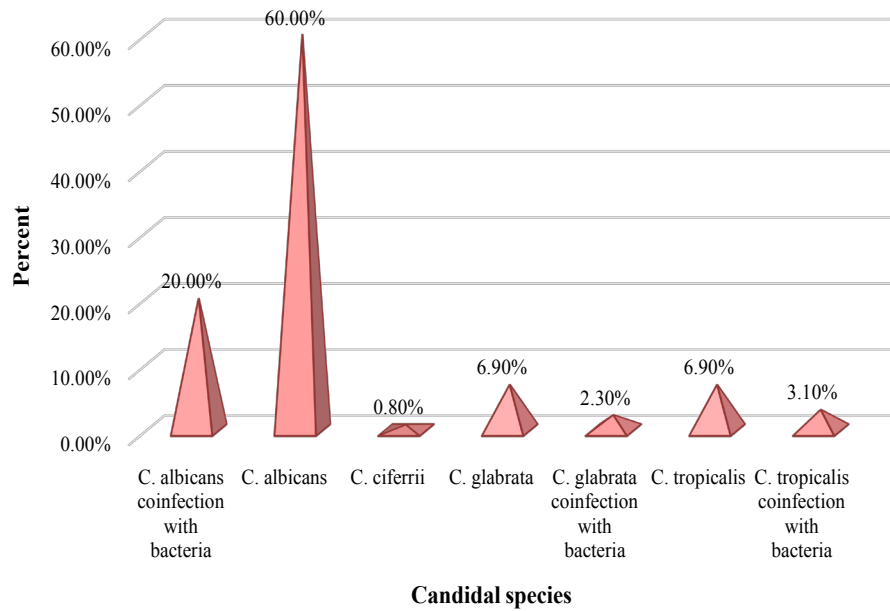


Figure 3. Bar chart showing frequency of Candidal species.

Table 4. Over all Sensitivity and resistance of clinical isolates to antifungal drugs.

Sensitivity pattern	Fluconazole		Voriconazole		Cross Resistance	
	n	%	n	%	n	%
Sensitive	55	42.3	38	29.2		
Resistant	75	57.7	92	70.8	66	50.7
Total	130	100	130	100		

Where, inhibitory zone > 14 mm of fluconazole (25 mcg) and > 17 mm of voriconazole (1 mcg) were considered sensitive [11] [12] [13].

Table 5. Distribution of death rate with gender in candidemia patients.

Gender	Candidemia patients				P value
	Alive 4 (50%)		Expired 4 (50%)		
	n	%	n	%	
Male 5 (62.5%)	1	20	4	80	0.028*
Female 3 (37.5%)	3	100	0	0	

*p value is significant (<0.05).

Table 6. Distribution of candidal species responsible for candidemia.

Candidal species	Frequency (n)	Percent (%)
<i>C. ciferrii</i>	1	12.5
<i>C. tropicalis</i>	7	87.5
Total	8	100.0

Table 7. Antifungal sensitivity profile of candidemia isolates.

Sensitivity pattern	Fluconazole		Voriconazole	
	n	%	n	%
Sensitive	3	37.5	1	12.5
Resistant	5	62.5	7	87.5
Total	8	100	8	100

Where, inhibitory zone > 14 mm of fluconazole (25 mcg) and >17 mm of voriconazole (1 mcg) were considered sensitive [11] [12] [13].

4. Discussion

Global rise in the occurrence of opportunistic fungal infections is exacerbating and has been quite challenging for the medical researchers to fix this issue with the development of new generation of antifungal agents. Since many of the currently available antifungal drugs have undesirable side effects, are ineffective against new or reemerging fungi and may lead to the rapid development of resistance. Furthermore, the improvement of new antifungal compounds is limited due to the eukaryotic architecture of fungal cell, which is very similar to the human cells, and has huge genome's plasticity [14] [15]. The knowledge of incidence and prevalence rate along with the resistance patterns of candidiasis in our country is lacking at national level which has a direct influence on treating these patients with the most appropriate antifungal drug [16]. Furthermore, antifungal susceptibility testing is not applied routinely as a standard protocol in most of the microbiological laboratories of Pakistan as the C/S testing is quite expensive, costs around 20 US \$ (dollars) that cannot be managed by majority of our low socioeconomic population [5].

Candidal patients notified in our study were from 2 month to 89 year of ages. Most of these patients had weak immunity, comorbidity, long term antibiotic treatments and hospitalization. Globally candidiasis is known as the disease of people at extreme of ages, usually occurs at the ages of less than 1 and greater than 70 year, particularly, due to their compromised immunity [17]. However, a study conducted in a tertiary setting of Rawalpindi, Pakistan showed candidiasis commonest in the age group of 21 - 40 year [5]. In comparison, most of our study population was from 51 - 60 year age group with mean age of 51 ± 20.27 year (Table 1). Two other local studies showed its high frequency in people at 50 year and another study at >60 year of ages [18] [19].

According to Figure 2, higher frequency of candidal isolates belonged to females, being 61.54% with female to male ratio of 8:5. Our results are parallel to the findings of two studies conducted in other cities of Pakistan (Islamabad and Rawalpindi), showed candidiasis predominantly in females (56% and 65.8%) [5] [17]. While contrary to our results, Bhattacharjee *et al.*, India, reported males (63.32%) more frequently infected with candidiasis than females (36.38%) [20].

In this study candidiasis was commonly isolated from vagina (26.9 %) followed by sputum (19.23%), urine (17.7%) and tracheal aspirates (13.1%) as

mentioned in **Table 3**. In comparison to our results a study conducted in Korea reported isolates commonly from urine 62.4% [21]. *Candida* is a common resident in areas of human body which are exposed to the environment therefore more prone to infect under advantageous circumstances as the patients' immunity decline, prolonged catheterization and pregnancy. Additionally, female reproductive hormones have receptors for candida due to which these organisms colonize and infect female genitalia (mainly vagina) [18] [22]. This is the reason why candidiasis was mainly isolated from females in our study.

In our study, majority of the candida positive samples were isolated from ICU patients with 40% frequency followed by patients admitted in wards and private rooms (36.2%) which is contradictory to the findings of Aslam *et al.*, Pakistan showed 52% of isolates, predominantly from Medical unit [5] and Noor *et al.*, Malaysia documented 25% from wards [23]. Previously it has been documented that Candidal strains are considered to be 80% responsible pathogenic opportunistic organism among hospital based fungal infections [20]. Prolonged hospitalization is associated with increased risk of acquiring candida infections as these patients usually hospitalized for their co-morbidities, had already poor health and weakened immune system [9].

In this study the principal pathogenic species responsible for candidiasis in general are *C. albicans* 80% followed by *C. tropicalis* 10%, *C. glabrata* 9.23% and *C. kefyr* 0.8% (**Table 3**). These findings are in accordance to a global surveillance study ARTEMIS conducted by Pfaller, expressed *C. albicans* as the main culprit among all other species with overall 57.7% prevalence internationally, after that *C. glabrata* 14.8% and *C. tropicalis* 9.4% were amongst the prevalent candida species [24]. Our results are also corresponding to a study of China by Park *et al.* showed *C. albicans* 64%, *C. tropicalis* 18.1% and *C. glabrata* 8.6% [16] [25]. While contrasting with the results of an Indian study which showed majority (63.3%) of the isolates were non albicans and the commonest isolated species was *Candida tropicalis* (41.1%), followed by *Candida albicans* (36.7%) and *Candida glabrata* (10%) [26]. Considerable discrepancy has been observed in the frequency of Candidal species distribution among all candida positive cases across the world but the actual reason is still unknown [10].

Azoles are one of the most commonly prescribed antifungal drugs for both systemic and topical fungal infections and are administered by oral and topical routes. These are frequently over the counter (OTC) available drugs, mainly fluconazole in pharmacies since 19th century and their constant rising resistance is becoming a big threat globally [27] [28] [29]. According to a global surveillance study, conducted from the year 2001 to 2007 resistance to fluconazole reported 13.8% and voriconazole 6% [10]. While this current study displayed overall resistance of 64.25% for both of antifungals, fluconazole and voriconazole while individual resistance for fluconazole was 57.7% and for voriconazole was 70.8% (**Table 4**), in contrast a study conducted in Rawalpindi, Pakistan showed overall resistance of both antifungals, 46% whereas, respective resistance to fluconazole

and voriconazole was 85% and 4% [30]. This shows a drastic variation in resistance patterns of widely prescribed, azoles in both cities of Pakistan, Karachi and Rawalpindi. The emergence of resistance to voriconazole is alarming as it is used as an alternative to fluconazole and itraconazole which may represent its irrational use more than fluconazole in our clinical settings. In our study cross resistance to both azoles observed was 66 (50.7%) among all isolates. While according to the previous studies conducted in various countries including Pakistan, Brazil and India, cross resistance between these antifungal drugs reported in a range of 34%, to 74% [18] [30] [31] [32], which is due to their analogous chemical structure [33].

Candidemia

Candidal infection in blood is a big life threat for the affected individuals. In this present study it is reported 6.15% which is resembling to the report of CDC (first global report published on candidemia) In this study, candidemia was found to be fairly prevalent in male gender 62.5%. The overall mortality rate observed was 50%, while in earlier studies death rate was found to be approximately 26% to 52% [34] [35] [36] [37]. According to reports of ARTEMIS and SENTRY, globally 38% to 70% candidemia occurs due to *C. albicans* among all species since centuries but now this rate is shifting towards non albicans [38] which is also obvious from our study, showing Non albicans 100% responsible for candidemia. These non albicans species in this study are *C. tropicalis* 87.5% as the main culprit of candidemia followed by *C. ciferrii* 12.5%. A study conducted in same country showed *C. tropicalis* 33% responsible specie for candidemia [39]. Unexpectedly, in our study a rare specie, *C. ciferrii* was isolated in a patient with blood stream infection. As documented, it is an infrequent candidal specie, known for its superficial pathogenicity in the field of microbiology [40]. This is in contrast to our study displaying *C. ciferrii* causing blood stream infection, which illustrates that any superficial candidal specie could invade systemically when person get immunocompromised which is also true for our study sample. Although our sample size for candidemia was small but these results verified the results of previous studies to consider *C. tropicalis* as the main culprit in cases of candidemia in our clinical set up. Our results are also in agreement with the previous researches showing non-candida albicans as the main source of invasive candidiasis [41].

In our study, for candidemia both antifungals were highly resistant but fluconazole showed more (37.5%) sensitivity in comparison to voriconazole (12.5%) (Table 5), among which *C. tropicalis* was 71.4% resistant to fluconazole and 100% to voriconazole. The global study ARTEMIS conducted from 1997 to 2003 showed resistance of *C. tropicalis* against fluconazole from 3% to 6.6% [15], which is quite low as compared to our results. Surprisingly our results were contradictory to our local data conducted in 2013, according to which the most resistant specie was *C. krusei* 100%, followed by and *C. glabrata* 15% [39].

5. Conclusions

Candidiasis is highly prevalent in our clinical set up and more frequently invading females in comparison to males as most of the positive isolates were retrieved from HVS (high vaginal swabs), while *C. albicans* was still found to be the most prevalent isolated specie among all positive candidal samples. We didn't find the shift towards non albicans except in candidemia cases where absolutely non albicans were the main offenders. Amazingly a rare candidal specie *C. ciferrii*, known for superficial candidiasis was reported in our study as a cause of systemic (nosocomial) blood infection.

Our study also demonstrated that the resistance of the most commonly prescribed antifungals, azoles have shown a rapid rise. Therefore, it is recommended that before prescription of antifungal drugs the clinicians should routinely recommend culture and sensitivity testing of samples taken from candida infected individuals. This will help in prescription of more effective and appropriate antifungal drugs which will lower the development of their resistance in our clinical set up. Subsequently the effective antifungal therapy will reduce the repeated use of antifungal drugs, with decreased frequency of adverse effects, improvement in over health and cut down the cost of antifungal therapy on the pockets of the candidal infected patients. Consequently, there is an intense urge to introduce novel antifungals with better efficacy, less side effects, and should be affordable to the patients for prolonged therapy. Additionally, preventive measures should be taken to decrease the incidence of nosocomial candidiasis.

Limitations of the Study

In this study we tested the sensitivity patterns of only two commonly prescribed antifungals due to limited budget. Additionally, the sampling was conducted only in one major city of Pakistan, Karachi and could not include fungal infected patients of other regions, also because of the limited budget, and lack of access to their clinical set ups.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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