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Original Research Article

Prevalence of Azole Resistance in Various Candida Species in Various Clinical Specimens at Tertiary Care Hospital

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Abstract

Background: Candida infections are among the most common fungal infection affecting immunocompromised patients in various disciplines of the health care system. In recent decades, due to dramatic increase in invasive fungal infections and related more common use of the antifungal therapy, resistance to antifungal agents has become an issue. ***Objectives:*** To determine the prevalence of candida species in various clinical specimen & their resistance to azole group of antifungal agents. ***Materials & Methods:*** This study was conducted at tertiary care hospital during January 2014 to June 2015. Total 23,896 samples (urine, blood, high vaginal swabs, oropharyngeal swab, body fluid, corneal scrapping, etc) were received over study period. ***Result***: Out of total 8864 culture positive specimen, candida species were isolated in 644 specimens. Among total 644 Candida isolates, C.albicans is the most prevalent species (44%). Amphotericin B (99.6%) had the maximum rate of susceptibility amongst all the antifungal agents, followed by Voriconazole with 94.8% susceptibility. Fluconazole, Itraconazole & Miconazole were 73.3%, 73.44% & 74.22% susceptible respectively. ***Conclusion***: Azole derivatives represent one of the major groups of antifungal drugs used in clinical practice to treat fungal infections. Empirical usage of Fluconazole in immune-compromised patients appeared to have played a major role in rising emergence of non albicans species resistant to Fluconazole.

Keywords: Candida infection, Candida albicans, Antifungal agents, Azole Resistance, Immunocompromised patients.

INTRODUCTION

Candida spp. have been considered the fourth most common primary blood stream infection in United states and the seventh most common pathogens to cause the nosocomial infections during the last four decades [1]. *Candida* is Latin named derived from 'Candidus' meaning "White".

In twentieth century pathological entities such as onychomycosis, osteomyelitis, endocarditis, chronic mucocutaneous candidiasis (CMC) were described. A variety of factors are known to predispose both superficial and deeps seated candidiasis. It is generally accepted that the incidence of yeast infections has increased during the last decades due to immunosuppression (pathological or iatrogenic) and increase in the use of broad spectrum antibiotics, etc. *Candida albicans* is the most common cause of mucosal candidiasis [1].

The extensive use of antimycotic drugs, particularly azoles, for prolonged therapeutic courses has led to changes in the relative prevalence of various Candida spp. Recent reports indicating the possibility of innate or acquired resistance of a number of *Candida spp* to several antimycotic drugs has emphasized the importance of in vitro antimycotic susceptibility testing and has prompted toward the standardization of these procedures and their interpretation. Azole derivatives represent one of the major groups of antifungal drugs used in clinical practice to treat fungal infections in humans, including skin and vaginal infections in the general population, and more serious life-threatening invasive mycoses in severely immunocompromised patients. At the molecular level, different mechanisms contribute to resistance against azole antifungal agents, reviewed in [2, 3]. Mechanisms of resistance include Modification of the antifungal target, Over expression of multidrug drug efflux pumps, Alterations in sterol biosynthesis, Alterations in the target enzyme, Point mutations in the gene, etc.

MATERIAL AND METHODS

Sampling Method & Sample Collection

This study was conducted at tertiary care hospital during January 2014 to June 2015. Total

23,896 samples were received over study period. Out of which 8864 specimens were culture positive. A total of 644 *Candida* isolated from various clinical specimens (urine, blood, high vaginal swabs, oropharyngeal swab, body fluid, corneal scrapping, etc) were taken up from out patients and patients admitted into various wards and intensive care units.

Blood samples were received in automated blood culture bottles & all other specimens were received in sterile universal container.

A detailed clinical history was taken with regards to the age of the patient, sex, underlying disease/conditions, immune-deficiencies, diabetes mellitus, pregnancy, malnutrition, any ongoing treatment and co morbid conditions.

Testing Method

These specimens were processed for the isolation of *Candida* spp. using standard Mycology methods. Primary identification was done by direct smear examination of specimen which includes Gram stain & wet mount preparation followed by fungal culture. All blood samples were received in BacT/ALERT aerobic blood culture bottle (Biomerieux) & after being signal positive, were subcultured. All Urine specimens were received in sterile universal container & cultured on blood agar with calibrated loop (0.001 ml) by semi-quantitative method. Followed by inoculation on to SDA screw cap bottle with antibacterial agent (Chloramphenicol) and incubated at 37°C and 25°C for 48 – 72 hours. Rest all specimens were directly cultured on SDA slant. The SDA slant was observed for growth, texture, pigment production and colony morphology. The various species were distinguished from each other based upon a combination of morphological and biochemical criteria.

The antifungal susceptibility testing was performed by disc diffusion method commercially available discs & MIC was done by Epsilometer test using E strip of each isolate with different anti fungal drugs as described in CLSI document M44- A (2).

Statistical Analysis

It was done using CDC Epi info version.7 software. The p value was calculated and a p value of less than 0.05 was considered statistically significant.

RESULTS

Out of total 23896 clinical specimens, 8864 positive were culture positive. From various clinical specimens, 644 Candida were isolated over the study period from out patients & patients admitted into various wards as well as intensive care units.

Out of 644 candida isolates, prevalence of various species of candida in clinical specimens was as below (Table-1).

Table-1: Distribution of Candida species- specimen wise

SL. No	Nature of specimen	Number of *Candida* spp. n= 644	*Candida* spp. identified					
			C.albicans	*C.tropicalis*	*C.krusei*	*C.parapsilosis*	*C.kefyr*	*C.glabrata*
1.	Urine	421	187	184	21	16	2	11
2.	Blood	182	73	27	41	17	1	23
3.	High vaginal swab	20	10	2	3	1	2	2
4.	Oropharyngeal swab	15	12	3	-	-	-	-
5.	Body fluid	5	1	3	1	-	-	-
6.	Corneal rim	1	1	-	-	-	-	-
	Total	**644**	**284**	**219**	**66**	**34**	**5**	**36**

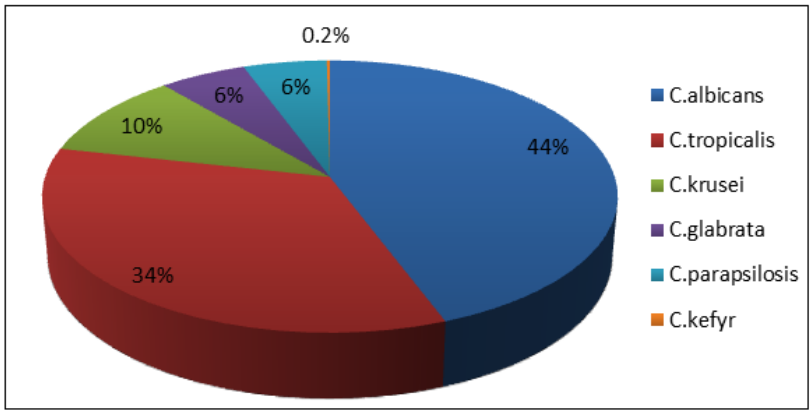

Fig-1: Distribution of different species of *Candida*

Among total 644 Candida isolates, C.albicans is the most prevalent species (44%).

Table-2: Age wise distribution of candidiasis

Age group in years	Number of isolates
0-9	239
10-19	34
20-29	75
30-39	80
40-49	68
50-59	54
60-69	55
70-79	27
>80	12

The most common age group involved is 0-9 yrs. (37.1%) (P value=0.001).

Table-3: Sex distribution of candidiasis

Sex	Candida positive isolates	Candida negative Isolates	Total
Male	350	5234	5584
Female	294	2986	3280
Total	644	8220	8864

Males are encountered more frequent than females (1.1:1) (P value=0.001).

Table-4: Distribution of predisposing factors in patients with Candidiasis

SL No	Predisposing factors	No. of patients
1	Indwelling device	207
2	Prolonged antibiotic therapy	186
3	Immunocompromised state	111
4	Diabetes mellitus	90
5	Neonates	62
6	Pregnancy	12

Indwelling device is the most common risk factor associated with Candida infection (32.1%) (P value=0.014).

Amphotericin B (99.6%) had the maximum rate of susceptibility amongst all the antifungal agents, followed by Voriconazole with 94.8% susceptibility. Fluconazole, Itraconazole & Miconazole were 73.3%, 73.44% & 74.22% susceptible respectively (Figure-2). *All the species (C. albicans, C. tropicalis, C. krusei,* and *C. kefyr*) were all highly susceptible to each of the echinocandins, except C. parapsilosis & C.glabrata.

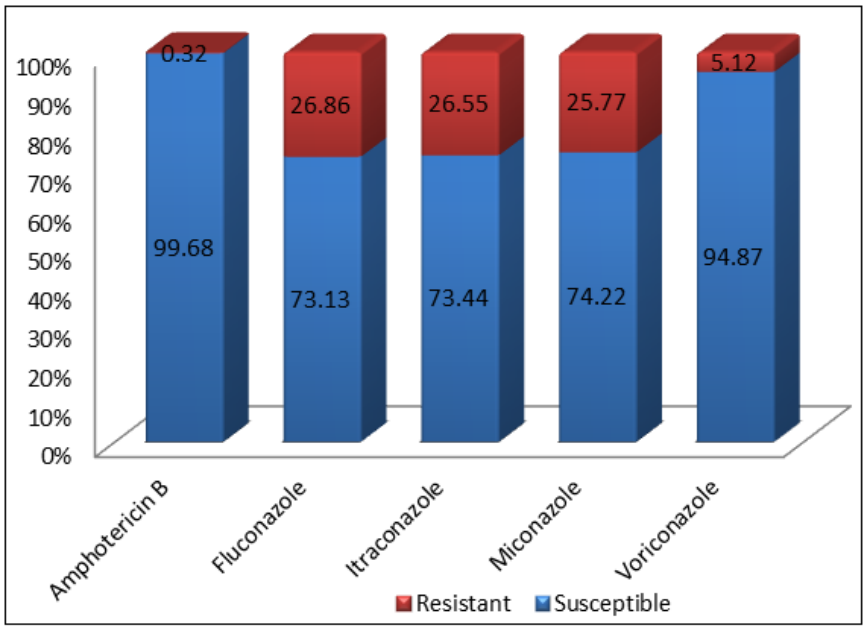

Fig-2: Antifungal susceptibility pattern of all *Candida* isolates

DISCUSSION

Hospital acquired infections is a frequent complication among patients admitted to tertiary hospitals. Patients with conditions as critically ill or weakened immune systems are at most risk. Infections with fungal pathogens, of which *Candida* species predominate, are an important cause of morbidity and mortality among the critically ill. In particular, the incidence of candidiasis has been increasing during the past years.

More than 17 species of *Candida* species have been implicated in human infections till date and the list of reported species continues to grow [4]. Although *Candida albicans* is the most frequently encountered organism, a number of reports have documented non-albicans *Candida* species as emerging pathogens in recent years. Our study reaffirms the shift towards non-albicans *Candida spp*.

In the present study six species of *Candida* were characterized using standard conventional methods. *Candida albicans* was the predominant species (44.3%). The following non-albicans species were isolated - *Candida* tropicalis (34.2%), *Candida krusei* (10.3%), *Candida glabrata* (5.6%), *Candida parapsilosis* (5.4%) *Candida kefyr* (0.2%).The overall isolation of non- albicans species was 55.7%.

Our study results were correlated with other reference studies shows *Candida albicans (*44%), the most common species isolated in all specimens. It also shows prevalence of *Candida tropicalis* which was the most common species among Non *Candida albicans*. In India various studies show 35-40% prevalence rate of *Candida albicans* and 60-65% prevalence rate of Non *Candida albicans* species [5-9].

The frequency of invasive mycoses has increased dramatically during the past two decades owing to medical advances such as intensive cancer therapy, broad-spectrum antimicrobial therapy, invasive medical devices, organ transplantation, human immunodeficiency virus (HIV) disease epidemic and an expanding aging population.

Erna M *et al.,* in their study observed that Candida infections of the urinary tract are strongly associated with the presence of urinary catheter [9]. Mauricio Carvalho *et al.,* observed that the presence of indwelling catheter favors the development of candiduria [10]. In our study most common associated risk factor was indwelling device (32.1%) (urinary catheterization, central venous line, mechanical ventilation, etc). A study conducted by Patel *et al.,* recorded a male preponderance, with an overall male: female ratio being 2:1 [8]. In our study also male: female ratio is 1.1: 1.

In our study, the antifungal susceptibility patterns revealed that Amphotericin B and Voriconazole has excellent in vitro activity overall against *Candida* species with 99.6% and 94.8% susceptibility respectively. In our study all *Candida* isolates show 26.5% resistance to Fluconazole, 26.8% resistance to Itraconazole, 25.7% resistance to Miconazole, 5.12% resistance to Voriconazole and only 0.3% resistance to Amphotericin B.

Table-5: A comparative study of azole resistance in all *Candida species* isolates

Study	Prevalence azole resistance in all *Candida species* isolates
Present Study	Fluconazole (26.5%) Itraconazole (26.8%) Voriconazole (5.12%) Amphotericin B (0.3%)
Shivanand Dharwad *et al.,* Karnataka 2011 [11].	Fluconazole (32%) Itraconazole (20%) Voriconazole (16%) Amphotericin B (8%)
Jaswinder Kaur Oberoi *et al*., New Delhi, 2012 [12]	Fluconazole (21.2%) Itraconazole (45.7%) Voriconazole (11.4%) Amphotericin B (10.4%)
Lata R Patel *et al*., Ahmedabad 2012 [8]	Azole (25%) Amphotericin B (100%)

CONCLUSION

The resistance to Fluconazole is of great concern because it is the most common azole used for treatment of disseminated candidiasis including candidemia. It is available in both intravenous and oral formulation with high bioavailability and is more cost effective than other antifungal agents. Thus, there is a great need for systemically active agents with potent fungicidal activity against Fluconazole resistant species.

Although Amphotericin B is effective against most strains of Candida species, it is not the first drug of choice for the treatment of candidemia because of nephrotoxicity associated with it. Caspofungin and other Echinocandins have been shown to exhibit potent

activity against Fluconazole-resistant *Candida* spp. To reduce morbidity and mortality caused by candidiasis, better methods of prevention such as Optimal use & care of indwelling devices, Prudent use of antimicrobial agents, Stringent infection control practices, Improved hand hygiene, etc. are important.

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