ORIGINAL RESEARCH PAPER

Volume-9 | Issue-3 | March-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CANDIDA SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN FROM CLINICAL SAMPLES IN A TERTIARY CARE CENTRE.

Microbiology

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ABSTRACT

INTRODUCTION: Candida can cause life threatening invasive to non life threatening mucocutaneous diseases. It is a known commensal that colonizes skin, gastrointestinal tract and reproductive tract. The pathogenesis and prognosis of candidial infections are affected by the host immune status and they differ greatly according to disease presentations.

MATERIAL METHODS: 104 Candida isolates from various clinical specimens over a period of 6 months were identified by standard laboratory protocols and their antifungal susceptibility was done.

RESULTS: Candida species isolated were C.albicans – 54, C.tropicalis – 35, C. krusei – 09, C. glabrata & C. dubliensis – 03 each. All the species were highly sensitive to Nystatin and Itraconazole, moderately sensitive to Ketoconazole, Fluconazole and Amphotericin B.

DISCUSSION: The present study highlights the speciation of candida species by conventional techniques along with CHROM agar and increase in resistance to various antifungal agents amongst Candida albicans and NAC group.

KEYWORDS

INTRODUCTION:

Infections caused by yeast like fungi belonging to the genus Candida are increasingly reported in recent years. Candida species is unique among mycotic pathogens because it causes broad spectrum of clinical manifestations ranging from mucocutaneous over growth to life threatening systemic infections.[1] The incidence of Candidiasis has increased in the recent years due to wide spread use of broad spectrum antibiotics, increasing numbers of HIV infected individuals, use of immunosuppressants, indwelling devices and other immunocompromised states, have further attributed to the higher rates of invasive Candidiasis.[2]

Various changes in the medical & surgical management of patients over the last few decades have helped fungi to emerge as a major pathogen. Amongst disseminated mycoses candidiasis remains the most prevalent.[3] Candidiasis is technically known as Candidiasis, moniliasis and oidiomycosis.[4] The yeast can cause life threatening invasive to non life threatening mucocutaneous diseases. Candida is a known commensal that colonises skin, gastrointestinal tract and reproductive tract. The pathogenesis and prognosis of candidal infections are affected by the host immune status and they differ greatly according to disease presentations. The pathogenecity of Candida is attributed to certain virulence factors such as ability to evade host defenses, adherence, biofilm formation and production of tissue damaging hydrolytic enzymes such as proteases, phosphorus and hemolysins.[5]

Presently there is an increase in infections by yeast that are resistant to antifungal drugs which are used worldwide thus use of in vitro laboratory tests for antifungal susceptibility may aid in choosing appropriate therapy for such infections. C. albicans is generally considered as most pathogenic member of genus and most common causative agent for different types of candidiasis.

In recent years variety of research from around the world has docum ented a shift from pervasive C. albicans to cryptic non albicans Candida species (NAC), these are closely related to C.albicans and cause similar clinical manifestations but differ with respect to epidemiology, virulence factors and susceptibility patterns to antifungal drugs.[6,7]

NAC is a heterogenous group of candida species which has 19 species implicated in human infections. C.tropicalis, C.krusei, C.glabrata & C.parapsilosis are the ones commonly reported. C. krusei is innately resistant to fluconazole and amongst C.glabrata strains 20% of strains acquire resistance during the course of therapy.[6,8] Recent studies have documented emergence of fluconazole resistance in the NAC species. C.parapsilosis is reported to have higher MICs to echinocandins the recent addition to antifungal agents.[9,10,11]

Emergence of NAC species causing human infections has highlighted the importance of identification of these species. These fungi vary in their susceptibility patterns thus it is important to identify and perform the sensitivity to the commonly used antifungal drugs which is helpful for early diagnosis and effective therapeutic outcomes.[12] As these species vary in their prevalence as per country and health care settings within the countries their identification and susceptibility pattern plays an important role in formulating of local therapeutic guidelines, enabling the physicians to be aware of available treatment options.[13]

In the present era of automation, laboratory medicine too has undergone rapid progression from conventional methods to rapid commercial systems to molecular diagnostics but all of this comes with a major drawback of high costs thereby limiting the use of such tests / systems for a limited / selected few. Conventional methods and techniques still are the main stay of species identification for most Candida isolates in microbiology laboratories till date.

MATERIALS & METHODS:

104 Candida isolates from various clinical specimens over a period of 6 months were identified by standard laboratory protocols and their antifungal susceptibility was done. Identification of the isolates was done by Dalmou technique, germ tube test, growth with color on CHROM agar. Antifungal susceptibility to drugs Amphotericin B, Nystatin, Itraconazole, Ketoconazole and Fluconazole was on Mueller Hinton agar supplemented with 0.5% methylene blue and 2% glucose. All the isolates were identified and their susceptibility pattern was noted.

RESULTS:

104 Candida isolates were identified from various clinical samples sputum – 47, urine – 23, high vaginal swab – 14, pus – 12, bronchoalveolar lavage – 4 and endotracheal secretions – 2, all isolates were identified as pathogens in the given samples.

The samples were collected across various age groups in the study, 10 samples were from children and 94 were adults. Age wise distribution of the samples 1 day to 14 years 10 samples received and 21 – 84 years 94 samples were received. Majority of the patients belonged to 61-84 years age group – 31 patients. The male female ratio was 4: 6.4 in the present study.

The various Candida species isolated were from various samples were C.albicans – 54, C.tropicalis – 35, C. krusei – 09, C.glabrata & C.dubliensis – 03 each. The antifungal susceptibility was performed on supplemented MHA with 2% glucose & 0.05% methylene blue by disc diffusion method. **C.albicans** isolates were 90.74% sensitive to Nystatin, 87.03% sensitive to Itarconazole, 75.92% sensitive to Ketoconazole, 50% sensitivity to

Fluconazole & 37.03% sensitive to Amphotericin B. **C.tropicalis** isolates were 94.28% sensitivity to Nystatin, 80% sensitivity to Itraconazole, 77.14% sensitivity to Ketococnazole, 57.14% sensitivity to Fluconazole & 48.57% sensitive to Amphotericin B. **C.krusei** isolates were 88.88% sensitive to Nystatin, 77.77% sensitive to Itraconazole & Ketoconazole each & 55.55% sensitive to Amphotericin B all isolates were reported resistant to Fluconazole. **C.dubliensis** isolates were 100% sensitive to Nystatin & 66.66% sensitive to Itraconazole, Ketoconazole, Fluconazole & Amphotericin B. **C.glabrata** isolates were 100% sensitive to Nystain, Fluconazole, Ketoconazole & Amphotericin B, 66.66% sensitive to Itraconazole.

Table 1: Distribution of Clinical Samples from where Candida species are isolated.

Sr. No.	Samples	Number of Samples
1.	Sputum	47
2.	Urine	23
3.	Vaginal Swabs	14
4.	Pus	12
5.	BAL	04
6.	Blood	02
7.	E.T. Secretions	02
	TOTAL	104

Table 2: Age Distribution of Patients in whom Candida species are isolated.

Sr. No.	Age in Years	Number of Patients
1.	0 – 11 months	04
2.	1 year – 10 years	05
3.	11 years – 20 years	01
4.	21 years – 30 years	16
5.	31 years – 40 years	07
6.	41 years – 50 years	17
7.	51 years – 60 years	23
8.	61 years – 70 years	18
9.	> 70 years	13
	Total	104

Table 3: Candida species isolated amongst clinical samples.

Sr. No.	Species Isolated	Number (%)
1.	C. albicans	54 (51.92%)
2.	C. tropicalis	35 (33.65%)
3.	C. krusei	09 (8.65%)
4.	C. glabrata	03 (2.88%)
5.	C. dubliensis	03 (2.88%)

Table 4: Non-albicans Candida species isolated.

Sr. No.	Non Candida Albicans species	Number (%)
1.	C. tropicalis	35 (58.33%)
2.	C. krusei	09 (15%)
3.	C. glabrata	03 (5%)
4.	C. dubliensis	03 (5%)

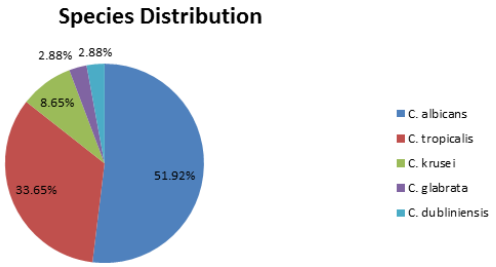

Table 5: Antifungal Susceptibility Test (S- Sensitive, R- Resistant)

Serial No	Organism	Nystatin %		Itraconazole %		Amphotericin B %		Fluconazole %		Ketoconazole %	
		S	R	S	R	S	R	S	R	S	R
1.	C.albicans	90.74	9.26	87.03	12.96	37.03	62.96	50	50	75.91	24.07
2.	C.tropicalis	94.28	5.71	80	20	48.57	51.42	57.14	42.85	77.14	22.85
3.	C.krusei	88.88	11.11	77.77	22.22	55.55	44.44	00	100	77.77	22.22
4.	C.dubliensis	100	00	66.66	33.33	66.66	33.33	66.66	33.33	66.66	33.33
5.	C.glabrata	100	00	66.66	33.33	100	00	100	00	100	00

DISCUSSION:

Candida species are known to cause life threatening systemic infections. The great concern over bacteriology has over shadowed mycology for the past few years. The rise in incidence of mycoses has given serious attention to the field of mycology and their laboratory diagnosis & interpretation. Recently three main families of antifungal agents are being used in clinical settings.

1. Polyene – Amphotericin B.
2. Azole derivatives – Itraconazole, Fluconazole, Voriconazole & Posconazole.
3. Echinocandins – Capsofungin, Micafungin & antidulafungins.[14]

The availability of new antifungal agents in recent years have provided clinicians with multiple options for treatment, prophylaxis, empirical and pre-emptive treatment. This increased use of selective antifungal agents has induced a selective pressure on fungal strains & resistance has emerged by secondary resistance or susceptible species have become resistant.[14] Thus the importance of species identification with their antifungal susceptibility pattern for better clinical diagnosis and therapeutic out come. The importance of species level identification has been recognized as Candida species differ in the expression of virulence factors and antifungal susceptibility.[15] Candida exhibit a direct impact on the choice of empirical therapy and clinical implications.

In the present study 51.92 % of C.albicans and 33.65% of C.tropicalis were isolated which is comparable to a study by Kanna BV et al where C.albicans isolated were 51% and C.tropicalis were 25%.[16] In another Indian study by Prasad RR et al C.albicans reported were 11.46%[17] Study from Kuwait by Mokaddas EM et al reported 39.5% in blood stream infections followed by C.parapsilosis at 30.6% & C.tropicalis at 12.4%[18] In a study by Golia S et al from southern India C.ablicans isolated were 36% followed by C.tropicalis 27%.[19] In another study by Rajeshwari PR et al from Southern India have reported 54% of C.albicans and 24% of C.tropicalis from vulvovaginal samples,[20] Candida albicans is overall considered most pathogenic member and the commonest cause of different types of Candidiasis. Recently many studies worldwide have documented shift from pervasive C.albicans to cryptic Non albicans Candida (NAC). NAC species are closely related to with C.albicans and cause similar clinical manifestations but differ in respect to epidemiology, virulence factors and in susceptibility patterns to antifungal agents.[6,7] NAC is a heterogeneous group of Candida species with 19/27species implicated in human infections. In present study the rates of NAC is 48.07% of which C.tropicalis is 33.65% followed by C.krusei 8.65%, C.glabrata and C.dubliensis at 2.88% each. Predominance of C.albicans has been seen in many studies and similarly increasing emergence of Non albicans Candida also has been documented by to 54.74. Amongst NAC, C.tropicalis has been reported as the predominant species which is comparable with the present study.[19,21,22]

In the present study disc diffusion method for antifungal susceptibility of all the Candida isolates was performed. C.albicans were highly sensitive to Nystatin, Itraconazole and Ketoconazole, 50% of these isolates were resistant to Fluconazole and 73% resistant to Amphotericin B. The sensitivity pattern of C.tropicalis shows very good sensitivity to Nystatin (94.28%), Itraconazole (80%), Ketoconazole (77.14%), these strains showed resistance to Fluconazole 42.86% and 51.43% resistance to Amphotericin B. C.krusei isolates were highly sensitive to Nystatin(88.88%) , Itraconazole and Ketoconazole (77.77%), resistance to Amphotericin B was 44.45%, all strains were reported resistant to Fluconazole. C.dubliensis isolates were highly sensitive to Nystain (100%), moderately sensitive to Itraconazole & Ketoconazole (66.66%), but 66.67% strains were resistant to Fluconazole & Amphotericin B. C.glabrata strains were highly sensitive to Nystatin, Fluconazole, Ketoconazole & Amphotericin B (100%) and moderately sensitive to Itraconazole (66.66%). In a study by Prasad RR et al[17] and Fadda et al[23] C.albicans have decreased sensitivity to azoles which is in accordance

with present study. Similar resistance to azoles is reported in various other studies.[18] In a study by Deorukhkar et al Fluconazole resistance was 19.07 % where as in present study resistance to Fluconazole is much higher at 46.15%. High resistance to Fluconazole is a major con cern as it is the most common azole used in the treatment of disseminated Candidiasis, being available in both oral & intravenous formulations, having high bioavailability and being most cost effective as compared to other antifungal agents.[24] Some studies have reported lower resistance and some have reported very high resistance in azoles (fluconazoles).[25,26,27,28,29] Higher resistance to Amphotericin B (55.76%) has been reported in present study as compared with Deorukhkar et al who have reported resistance to Amphotericin B at 4.63%[24] though Amphotericin B is not the first drug of choice for candidemia due to nephrotoxicity associated with the drug.

The present study highlights increase in resistance to various antifungal agents both in Candida albicans and NAC group thus it reiterates the fact that speciation and antifungal susceptibility should be performed for all Candida isolates for better clinical and therapeutic results and for epidemiological purposes.

SUMMARY & CONCLUSIONS:

THE PRESENT STUDY CONCLUDES:

1. Candida infections are on the rise due to various factors. It is important to understand every aspect of Candida infections. Prompt and specific diagnosis of such infections is the need of the hour.
2. Emergence of Non Candida albicans species as causative agent of infections thereby highlighting the importance of species identification for all Candida isolates.
3. Characterization of infecting Candida strains to species level helps to recognize disease patterns by various species and also to identify the intrinsically resistant species which is an important tool for selection of appropriate therapeutic antifungal agents.
4. Commercial and molecular diagnostic methods are rapid and reliable but due to higher cost their use is limited. Present study uses conventional techniques for identification of all species thus re-enforcing the importance of conventional methods of identification in an era of automation, though in some cases automation is more helpful as a diagnostic tool and their impor tance cannot be over ruled.
5. Conventional methods have since long been used as a benchmark identification procedures for all Candida species. CHROM agar along with Dalmou technique is a simple and inexpensive method for identification of various Candida species in a resource limited setting. Major advantage of CHROM agar is that it provides the rapid isolation and identification of medically important Candida species.
6. Effective management of Candidiasis requires both early diagnosis to species level as each species varies in their susceptibility pattern and prompt initiation of antifungal therapy where ever needed thus decreasing patient morbidity & mortality. It helps to reduce the empirical use of antifungal agents which is a common practice today.

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