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To study the incidence and control of *Candida albicans*, in immune compromised individuals

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ABSTRACT

Candida albicans is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections, *Candidal onychomycosis*, an infection of the nail plate in humans. Systemic fungal infections (fungemia) including those by *C. albicans* have emerged as important causes of morbidity and mortality in immune compromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation. *C.albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns

Keyword: Opportunistic pathogen, immune-compromised host

INTRODUCTION

This disease usually occurs during the first year of life or is seen in patients with immune system dysfunction. It affects both males and females and may be associated with an inherited defect of the cell-mediated immune system that allows auto antibodies to develop against target organs. Immunosuppressive conditions result due to treatment with antibiotics and lead to eliminating the yeast's natural competitors for resources. In such immune compromised patients, *Candida* spp. can affect the oesophagus with the potential of becoming systemic, causing a much more serious condition-Candidemia.

The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for Candida species to invade tissues normally resistant to invasion. Nearly 70-80% of Candida species are isolated from the respiratory secretions of mechanically ventilated patients. *Candida glabrata* and *Candida tropicalis* account for approximately 5 to 8% of isolates, while other non-albicans Candida species occur rarely.

Widespread usage of antifungal has rapidly led to the increasing cases of drug resistance which emerges as a threat to the antifungal therapy and therefore there is an urgent need for novel therapies against this pathogen. It is observed that 60-90% of the patients have some underlying immune suppressive condition such as chronic steroid use, solid organ transplantation, malignancy or human immunodeficiency virus (HIV) infection. These patients are in a state in which the immune system's ability to fight infectious disease is compromised or entirely absent and become vulnerable to opportunistic infections, in addition to normal infections that could affect everyone (Arendrup, 2013).

Candida species are frequently part of the human body's normal oral flora. Candidiasis is an infection caused by species of the genus Candida, predominantly *Candida albicans*. This disease usually occurs during the first year of life or is seen in patients with immune system dysfunction. It affects both males and females and may be associated with an inherited defect of the cell-mediated immune system that allows auto antibodies to develop against target organs. Immunosuppressive conditions result due to treatment with antibiotics and lead to eliminating the yeast's natural competitors for resources. In such immune compromised patients, Candida can affect the oesophagus with the potential of becoming systemic, causing a much more serious condition-Candidemia.

The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for Candida species to invade tissues normally resistant to invasion. Nearly 70-80% of Candida species are isolated from the respiratory secretions of mechanically ventilated patients. *Candida glabrata* and *Candida tropicalis* account for approximately 5 to 8% of isolates, while other non-albicans Candida species occur rarely. Oropharyngeal candidiasis is also seen frequently among HIV-infected individuals, particularly those with more advanced immune suppression (Barelle et al., 2006).

Candidiasis is an infection caused by species of the genus Candida, predominantly *Candida albicans.* The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for Candida species to invade tissues normally resistant to invasion. This disease usually occurs during the first year of life and in patients with immune system dysfunction. It affects both males and females and may be associated with an inherited defect of the cell-mediated immune system that allows auto antibodies to develop against target organs.

The frequency of invasive mycoses due to opportunistic fungal pathogens has increased significantly over the past two decades (Hajjeh et al., 2004). This increase in infections is associated with excessive morbidity and mortality and is directly related to increasing patient populations at risk for the development of serious fungal infections, which includes individuals undergoing solid-organ transplantation, blood and marrow transplantation (BMT), and major surgery and those with AIDS, neoplastic disease, immunosuppressive therapy, advanced age, and premature birth (Pfaller et al., 2004). Serious life-threatening infections are being reported with an ever-increasing array of pathogens, including the well-known opportunists *Candida albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus* (Mathew et al., 2009; Giri et al., 2014).

Objectives

- To study the incidence of this opportunistic pathogen in individuals.
- Identification of the etiological agent form samples collected from individuals.
- To study the Anti-bio gram pattern of these isolates against conventional antibiotics

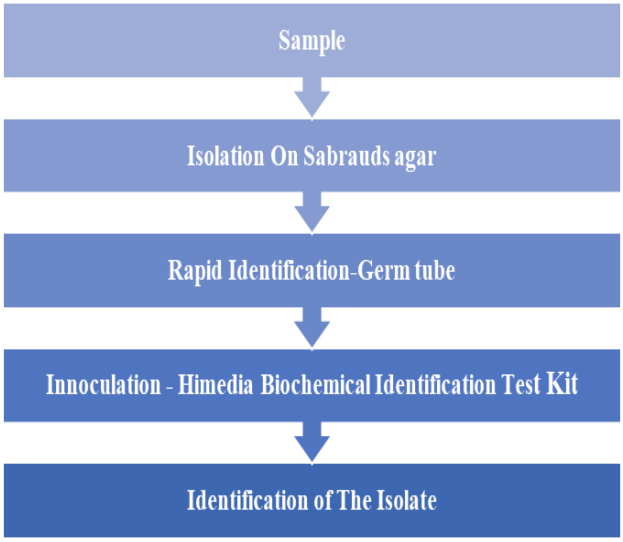
MATERIALS AND METHODS

Sample collection:

Oral swabs were collected from individuals, after noting their brief infection history along with the past antibiotics administered.

Inclusion Criteria: Patients clinically suspected to and showing suspicion of infective etiologic and prolonged antibiotic treatment that were able to produce a brief, relevant history.

Exclusion Criteria: Samples from healthy individuals and not administered with antibiotics was rejected.



Flow sheet for sample processing

A wet mount of a small amount of sample in a liquid medium was prepared using either of the two liquids. Normal saline is a physiologic solution, so cell membranes are preserved, and vital activities remain undisturbed or Potassium hydroxide (KOH) dissolves cell membranes and other biologic materials, but not the cellulose found in the cell walls of fungi, everything except the Candida. This property makes it particularly useful in identifying Candida. The analytical profile index or API system was used for quick identification of clinically relevant isolates after a prior confirmation. Incubating the strips at 37^0 C for 24 hours and using the appropriate reagents the isolates were identified up to the generic level.

KB006 Hi-media is a standardized colorimetric identification system utilizing twelve conventional biochemical tests that can be used for identification and differentiation of Candida species. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated by a spontaneous colour change in the media.

Antibiotic sensitivity Testing: Disc diffusion test a qualitative test method documented by The National Committee for Clinical Laboratory was used to test the antibiotic sensitivity (NCCLS). This was carried out using the Kirby Bauer's technique

RESULTS AND DISCUSSION

All Candida isolates were found to be susceptible to amphotericin B. Fluconazole and ketoconazole resistance was seen in (5 strains) 29.4% and (2 strains) 11.7% of Candida isolates, respectively.

Increase in fungal pathogens 17 % was observed in the present study. Invasive fungal infections limited therapeutic options and emergence of MDR together build a burden on patients with compromised immunity. Due to increasing prevalence in various patient groups, Candida spp. has gained remarkable importance and among all Candida spp., *Candida albicans* was most frequently isolated from respiratory samples of affected patients who had administered prolonged antibiotic treatment. *Candida alibicans* along with candida tropicalis were predominantly found in the hospital set presence of pseudo mycelia was an important feature associated with the fungi. Although the antifungal drugs used in clinical treatments appear to be diverse and numerous, only few classes of antifungal agents are currently available to treat mucosal or systemic infections with Candida spp. Although the antifungal drugs used in clinical treatments appear to be diverse and numerous, only few classes of antifungal agents are currently available in oral and intravenous forms which can be administered in immune compromised patients. Additionally, antifungal resistance based on different mechanisms continues to grow and evolve and exacerbate the need of new treatments against Candida infections. Fluconazole can also be effective against some non-albicans Candida species, including *Candida parapsilosis*, *Candida tropicalis* and *Candida glabrata*, although higher doses may be required

Table 1: Identification of clinical isolates

Clinical isolates	No.	Clinical isolates	No.
Klebsiella	16	Micrococci	5
Pseudomonas	12	E. coli	2
Streptococcus pyogenes	10	Corynebacterium diphtheriae	0
Candida albicans	**17**	Proteus Mirabilis	1
Acinetobacter	5	No Growth	20
Staphylococcus aureus	12	Total	100

Table 2: Details of samples

Identification of Candida spp using KB006 Hi-media is a standardized colorimetric identification system

ISOLATES	C1	C2	C3	C4	C5	C6	C7	C8	C 9	C10	C11	C1 2	C13	C14	C15	C16	C17
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	+	+	+	+	+	+	V	+	+	+	+	+	+	+	V	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-
Trehalose	+	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+
	Ca	Ca	Ca	Ca	Ca	Ct	Ct	Ca	Ca	Ct	Ca	Ca	Ca	Ca	Ct	Ct	Ca

Key: Ca : *C.albicans* **Ct:** *C.tropicalis* **'+' :**positive test result **'-':**negative test result

Table 3: Standard biochemical results for Candida spp

	UREASE	MELIOBIOSE	LACTOSE	MALTOSE	SUCROSE	GALACTOSE	CELLOBIOSE	INOSITOL	XYLOSE	DCITOL	RAFFINOSE	TREHALOSE
C.albicans	-	-	-	-	+	+	-	-	+	-	-	+
C.tropicalis	-	-	-	+	-	-	-	-	-	-	-	+
C.dubilensis	-	-	-	+	+	+	-	-	+	-	-	+
C.glabrarta	-	-	-	+	-	-	-	-	-	-	-	-

Table 4: Microscopy of the samples

SAMPLE	MICROSCOPY			
C1	EC+ PC+		C 9	Oval yeast cells
C 2	PC<10 EC <10 PSEUDO HYPHAE		C 10	Oval yeast cells
C 3	Oval yeast cells		C 11	Oval yeast cells
C 4	PC10-12/EC>25		C 12	Oval yeast cells
C 5	Oval yeast cells		C 13	Oval yeast cells
C 6	PC++/PSEUDOMY		C 14	Oval yeast cells
C 7	PC<25 EC>10		C 15	PC+EC
C 8	PC<10 EC <10 PSEUDO-HYPHAE		C 16	PC<10 EC <10 PSEUDO HYPHAE
			C 17	PC<10 EC <10 PSEUDO HYPHAE

Antibiotics testing: Disk diffusion method. Strains were tested for

1. Amphotericin B. 2.Fluconazole 3. Ketoconazole

Search for novel therapeutic alternatives has emerged due to clinical needs for novel antifungal agents with broad spectrum activity and minimal toxic effects on the host. Herbal drugs have made their importance felt in the last few decades whose prevalence is continuously increasing in both developing and developed countries due to their natural origin and lesser side effects. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief and needs to be exploited.

These extracts in treating these opportunistic infections can prove to be useful in patients with low immune response, especially those which have been overdosed with heavy third generation antibiotics for treating other ailments.

The present study provides insight into the occurrence of candida species as a competent and common opportunistic pathogen in immunocompromised host. As antibiotic usually comprises a single chemical component, whereas herbal extracts comprise of a large number of chemical components. These multiple components with multiple targets are challenging for microorganisms to develop a resistance to and therefore, these extracts are a better choice for treatment. Potential of herbs can be exploited in treating these infections rather than using further antibiotic treatment.

Conflict of interest

The author declares that there is no conflict of interest.

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