STM JOURNALS

Research & Reviews: A Journal of
Immunology

ISSN: 2277-6206 (Online)
ISSN: 2349-1280 (Print)
Volume 12, Issue 2, 2022
DOI (Journal): 10.37591/RRJoI

http://stmjournals.com/index.php?journal=RRJoI&page=index

Research RRJoI

Antifungal Susceptibility Pattern of Candida Species Isolates from Dehradun City Hospitals

Manshi Gusain*

Abstract

The present investigation on the topic entitled "Antifungal susceptibility analysis of Candida species isolates from Dehradun City hospital." was accomplished in the Doon University Microbiology laboratory (P.G.) and Hospital, Dehradun, Uttarakhand, during the period of February 2021 to July 2021. A total of 42 clinical samples were collected from patients, suspected of candidiasis from Dehradun city hospitals. Clinical samples were collected from oral cavity, blood, vaginal fluid and urine of the patients. Among them 24 yeasts were obtained. These were identified and characterized by germ tube formation, Microscopic, morphological and biochemical test. Out of 24 Candida isolates, Candida albicans was the commonest 16 (66.66%). Other non albicans species were 8 (33.33%). Out of 8 non albicans species 5 (62.5%) were C. tropicalis and 3 (37.5%) were C. glabrata. Prevalence of Candida in urine samples were found comparatively higher. We have studied 16 urine samples out of them 13 (81.2%) yeasts isolates representing 3 Candida species were found. The isolates 8 contained C. albicans as the most prevalent species (50 percent), after by C.tropicalis 3 (18.7%) and C. glabrata 2 (12.5%). We have conducted antifungal sensitivity test for 24 isolates of yeasts. According to CLSI recommendations, antibiotic sensitivity evaluation was carried out using Disc Diffusion (DD) method.Using fluconazole discs 8 (33.3%) of the 24 yeasts isolates were classified as S, 10 (41.6%) were S-DD and 6 (25%) were resistant. Using Itraconazole discs 6 (25%) of the 24 yeast isolates were classified as S, 10 (41.6%) were S-DD and 8 (33.3%) were resistant. Using Clotrimazole discs (10µg) 8 (33.3%) of the 24 yeast isolates were classified as S, 15 (62.5%) were S-DD and 1 (4.16%) were resistant. Using ketoconazole discs 23 (95.8%) of the 24 yeast isolates were classified as S, 1 (4.16%) were S-DD and No one was resistant. Using amphotericin B discs 14 (58.3%) of the 24 yeast isolates were classified as S, 5 (20.8%) were S-DD and 5 (20.8%) were found resistant. Candida species isolates showing 33.3%, 25.0% resistance against itraconazole and fluconazole respectively. Systemic infection due to yeast and resistance to antifungal is on rise in Indian population.

Keywords: Opportunistic Pathogen, Glossitis, cheilitis, *Itraconazole*, Chronic mucocutaneous.

***Author for Correspondence**
Manshi Gusain

Student, Department of Microbiology, Doon Paramedical College 28, Chakrata Road, Behind Bindal Petrol Pump, Paltan Bazar, Dehradun, Uttarakhand, India

Received Date: June 22, 2022
Accepted Date: July 27, 2022
Published Date: August 25, 2022

Citation: Manshi Gusain. Antifungal Susceptibility Pattern of Candida Species Isolates from Dehradun City Hospitals. Research & Reviews: A Journal of Immunology. 2022; 12(2): 12–29p.

INTRODUCTION

Candida As Pathogenic Yeast

Typical Candia Yeast Infection Symptoms are Sexual dysfunction, Suicidal depression, Arthritis, Autism Yeast strains are opportunistic pathogens that are common members of the human commensal bacteria. Yeasts have attracted growing interest among researchers due to the increased incidence of severe oral candidiasis. In the oropharynx, Candida albicans has been the most widespread cerevisiae genus Immunocompromised individuals are, however, prone to infections by otherwise rare yeast species, such as *Candida*

dubliniensis, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida kefyr, Candida glabrata, and *Candida guilliermondii*. [1] was the first who observed a fungus associated with thrush, but its identity was incorrect. [2] studied the organism isolated by Langenbeck but came to the conclusion that it was a species of *Sporotrichum*. A fungus that is mostly found associated with plant materials [3, 4]), but the organism described by [5] and others was morphologically different from that of *Monilia.* The controversy still persisted until [6] proposed that the organism of thrush lesions was clearly not a species of *Monilia* and, as a consequence, she proposed that the favoured generic name should be *Candida,* Other investigators attempted to classify the thrush agent by using biochemical and serological assays however, [7] focused her research on the use of more rigid criteria in the identification of the fungus, concluding that, of all the yeast like fungi, only *Candida albicans* was pathogenic for laboratory animals, although, she was not entirely correct in her concept [8].

Chronic hives, Fatigue, Irritability, Digestive disorders, Muscle pain, Short attention span,etc. Antifungal medicines, such as fluconazole and polytene amphoteric amphotericin B, are typically used to treat these infections. In aids patients, azole-resistant genotypes of Albicans are more prevalent. According to a recent study, up to one-third of all AIDS patients orally harbour an azole-resistant C. albicans isolate.

CANDIDIASIS TYPES

Types of candidiasis are as follow:

1. Candida Infection of the Throat

As a result of diabetes mellitus, cancer, neutropenia, and HIV infection, it is frequently linked to significant immunological impairment.

2. Candidiasis of the Skin

Inflamed thrush would be most typically found in the axillae, groyne, inter- and submammary folds is Common in infants exposed to unhygienic conditions of persistent moisture as a result of abnormally started changing unhygienic cloth nappies.

3. Chronic Candidiasis of the Mucosa

Chronic infections of the skin candidiasis is a type of persistent candidiasis of the skin, nails, and mucous membranes caused by Candida albicans that happens in patients with a variety metabolic abnormalities to cell-mediated immunity

4. Candidiasis Spinal Bifida and Neonatology

The main risk factors for diseases (e.g., in newborns are small for gestational age and maturity level, prolonged intravenous urinary catheter, and antibiotic use.

5. Candida Gastrointestinalis

Acute leukaemia and other haematological malignancies can cause numerous ulcerations in the stomach and, less frequently, the jejunum and small bowel.

DRUG RESISTANCE

The survival or progression of an infection in spite of successful pharmacological interventions is known as the drug resistance.

Development of Resistance to Antifungal Drugs

The progression of antibiotic resistance is dependent on genetic differences, the sole cause of which is mutated

Therefore, we have selected this problem to understand the impact of candidiasis in northeastern region of the Uttarakhand together with our major impact is on the analysis drug resistance in Candida albicans and non albicans Candida species. We have considered most popular drugs fluconazole, Itraconazole and Amphotericin B used as threpatic agent for candidiasis in this region.

Aspects of Candida Infection

In the summer, and more frequently in girls than males, is candida infection. Some factors that do influence carriage however include salivary factors, temporal variations, smoking and related factors, immune status and *Candida* carriage in hospitalized patients [8,9].

Oral Defences Against Candida Infection.

Salivary Nonimmune Factors

a. Iron: Iron is an important nutrient for both fungi and bacteria. [10]. Lactoferrin and other iron-binding proteins have been demonstrated to stop C. albicans' development or even kill it [11].
b. Lysozyme: Polymorphonuclear leukocytes, saliva, gingival crevicular fluid, and lysozyme (muramidase), a low-molecular weight protein, are all found in rather high concentrations. By increasing permeability, lysozyme can harm Candida *albicans are* the least sensitive to lysozyme.
c. Salivary histidine-rich polypeptides (HRPs) are antifungal and reduce Candida levels in healthy individuals along with other antimicrobial proteins such lysozyme [12].
d. Lactoferrin: During inflammation of the oral mucosa and parotid gland, salivary lactoferrin concentrations rise drastically. It is also present in polymorphonuclear leukocytes and parotid and submandibular saliva. Its antifungal properties are thought to be a result of ironet binding [13].
e. *Salivary peptides:* Salivary protein that are epitopes similar to fully functional surface antigens (blood group antigens) influence competition and limit surface adherence of bacteria to the mucosal surface, which enhances the cleansing action of mouth [14].

Immune Mechanisms in Host Resistance To Candidiasis

a. *Granulocytes:* White blood cells, which are likely bone marrow-derived cells, are essential to the body's built-in defence against Candida albicans. [15] described a case of alveolar pyogenic granuloma in the maxilla produced by infection with C. albicans in a patient with MPO deficit. the condition appears to be caused by the myeloperoxidase (MPO)-hydrogen peroxide-halide system [16]
b. *Cell-mediated immunity:* Although phagocytosis is the main mechanism for controlling Candida albicans, the inherent candidacidal abilities of both granulocytes and macrophages are quite limited, and full expression of their effect requires augmentation by cytokines synthesised induced by T-Cell [17]. In this perspective, it is notable that Candida infection significantly enhances the expression of HLA-DR and HLA-DQ antigens on the surface membranes of epithelial cells [18] and that human Candida-specific T lymphocytes require HLA-DR-compatible macrophages for their activation [19].
c. *Humoral immunity:* Serum antibodies may inhibit C. albicans growth [20] Saliva's main particular immunologic component, sIgA, may act as the body's first line of defence against oral candidosis by clumping together the organisms and preventing their adhesion to mucosal epithelium.

Candidal Species Risks: Various C. albicans biotypes and strains have different pathogeneses.

a. *Candidal enzymes:* It has been hypothesised that C. albicans creates a "endotoxin" [21], albeit the quantities of endotoxin discovered in vivo might not be enough to cause harmful consequences. Alternately, the organisms might create enzymes that make it easier for substances to penetrate mucosal membranes [22]; Candida can undoubtedly do this. Extracellular proteinases have also been connected to C. albicans' pathogenicity. Proteinase-deficient strains don't cause any harm, and the way they adhere also reveals how much secretory proteinase is expressed [23]. By acidic proteinases of Candida, particularly in low pH

circumstances, salivary proteins, including IgA, can be practically entirely destroyed.

b. *Variations in temperature:* C. albicans' virulence can also be affected by the temperature at which it is cultured [24]. Room-temperature-grown yeast is more resistant to polymorphonuclear leukocyte death [15].
c. *Candida adhesion:* Candida albicans' non-specific affinity for and binding to acrylic resin (caused by Candida-associated denture stomatitis) and other plastics may be a significant factor in its pathogenicity (catheter-related candidosis). Additionally, it has been proposed that interactions involving divalent cations may be involved in C. albicans' adherence to oral mucosal cells.

Candida infections in the mouth- Classification and Clinical Signs are described below.

(a) Vulvovaginal Candidiasis

It is thought that 75% of all women will encounter at least one episode of vulvovaginal candidiasis during their lifetime, and that almost half of them will experience numerous episodes [16]. Candida vaginitis is a frequent issue caused by an excess of the Candida species. Nearly half of all college-age women will have experienced at least one episode of Candida vaginitis by the age of 25 [17]. There are various species of Candida that can cause vulvovaginal candidiasis (VVC), but C. albicans is the most prevalent. The yeast most frequently found in vaginal fungal cultures is C. albicans. In HIV-infected women receiving treatment, the advent of C. glabrata vaginal colonisation has been recognised as a significant concern. Fluconazole prevention. Candida vulvovaginitis has also been linked to Human immunodeficiency virus a marker of progression toward AIDS, and the most frequent clinical manifestation of HIV infection [18].

(b) Albicans Vulvovaginitis and Sexual Diseases (STD

Albicans vulvovaginitis is not regarded a sexual transmission illness because it affects celibate women. As previously stated, Candida spp. are considered normal vaginal flora [19]. This is not to say that Candidiasis could be transmitted sexually. There is evidence in favour of sexual transmission, which includes: (1) Penile colonisation is four times greater in men's sex collaborators of Candida vulvovaginitis patients. (2) Infected partners frequently have identical strains.

RESISTANCE TOWARDS CANDIDIASIS

The complex manifestation of host and pathogen variables that causes drug resistance. Treatment resistance can be axiology as the continuation or advancement of an infection in spite of effective drug therapy.

Resistance Developing to Antifungal Drugs

All pathogenic organisms, including microorganisms, can develop drug resistance through an evolutionary process that is sparked by antimicrobial agent exposure [20]. Antimicrobial agents are rarely used in a way that completely eliminates the pathogen population, leaving survivors subject to natural selection. As a result, resistance develops. Antimicrobials are rarely used in a way that completely eliminates the pathogen population, leaving survivors subject to biological evolution. As a result, resistance develops. When the pathogen population is large enough, the emergence of resistance is all but certain to occur over the duration of therapeutic therapy. More than the range of possible modifications that provide the pathogen the ability to escape, destroy, or make inactive a treatment are responsible for the formation and spread of drug resistance. To evaluate the overall phenotype, such stress mutants interact with the entire genetic code. [21]. Reproductive output, or fitness, is a resistance phenotype's most crucial element in the evolutionary process. When a pathogen population is exposed to a drug, how rapidly resistance spreads and whether resistance will endure in the absence of the treatment are determined by the relative fitness of sensitive and resistant genotypes. Although the molecular and genetic causes of antifungal medication resistance are widely known, further research is needed to understand how these causes evolved and persisted in populations of pathogens.

Antifungal Drugs

Antifungal treatments against *Candida* infections are hampered by several factors, including the limited number of active agents, the emergence of refractory fungal species, and the development of resistance. Scientists involved in the design and isolation of antifungal medicines have mostly taken advantage of this characteristic as ergosterol production is only found in fungi and is essential for their proliferation. As a result, most of the antifungal agents used in medicine target the ergosterol biosynthetic pathway and include polyenes, azoles, allylamines, or morpholine derivatives. Among currently used antifungal agents, 5-FC is one of the few that does not directly interfere with the ergosterol biosynthetic pathway.

Polyenes

A class of the all antifungal substances called polyenes was first initiated in the early 1950s. Streptomyces nodosus produces amphotericin B, one of the most effective polyene derivatives [22] AmB is extremely insoluble in water and can generate dissolved salts both in basic and acidic conditions. It cannot be absorbed intramuscularly or orally. AmB is an effective antifungal drug, it possesses several drawbacks limiting its clinical utility. Systemic and renal problems are often encountered with AmB [3].

Azoles

The largest class of antifungal drugs, azoles, are synthetic chemicals that were first discovered in the late 1960sare artificial substances that are part of the largest class of antifungal agents. Triazoles and N-1 substituted imidazoles (such as ketoconazole, miconazole, and clotrimazole) are two types of azole antifungal medications used in medicine (fluconazole, itraconazole).The new generation of azole antifungal agents under development (posaconazole, ravuconazole, and voriconazole) belongs also to triazoles.

Determinants of Azole Antifungal Antibiotic Resistance

Reports on resistance to azole antifungal agents have been rare until the late 1980s. After lengthy treatment with miconazole and ketoconazole, C. albicans showed the first signs of resistance [23]. Yeasts can develop a variety of defence mechanisms against azole antifungal medications.. In clinical isolates resistance to azole antifungal agents can be the result of the following phenomena: (i) cells can fail to accumulate these agents; (ii) the cellular content of Erg11p can be elevated; (iii) the affinity of Erg11p to these agents can be decreased; (iv) the ergosterol biosynthetic pathway can be altered by inactivation of the sterol $\Delta^{5,6}$ desaturase.

MATERIALS AND METHODS

1. Collection of Clinical Samples

Clinical samples were collected from oral cavity, vaginal swab, and urine of the patients. The samples were collected in YEPD broth aseptically and kept in icebox to maintain their viability

2. Clinical Sample Handling

Swabs and sputum for microscopy and culture were examined because drying might impair the viability and morphology of yeasts and were kept in transport medium and stored in a refrigerator for a maximum period of 24 h in Sabouraud's dextrose agar (SDA) culture medium.

3. Isolation of Pure Culture

The samples obtained were aseptically inoculated on several non-selective and selective agar media including SDA and YEPDA Hi-Media with antibiotics (chloroamphenicol 20 µg/mL) and incubated at 37°C for 48 to 72 h to obtain discrete colonies and pure cultures.

Identification of Candida Species

For identification of yeast, isolates were first subjected to Gram's staining and examined under binocular microscope. Standard, methods for the identification of *Candida* species rely on a

combination of morphological and biochemical characteristics [24].

4. Growth on Hichrome Candida Agar

For the identification of yeast, isolates were directly streaked on HiCROME Candida differential agar. Further identification and characterization were done by morphological and biochemical test.

GERM TUBE PRODUCTION TEST

A tiny part of a pure colony of the test organism was injected using a sterile loop into sterile test tubes containing 0.5 mL test serum. The final mixture underwent a two-hour aerobic incubation at 37°C. A drop of the yeast-serum mixture was placed on a clean, plain microscope slide, covered with a coverslip (22 × 22 mm), and viewed under a microscope using the 10X, 40X, and 100X objective lenses at intervals of 10 minutes. The development of germ tubes was verified by the presence of tiny filaments extending from the cell surface. This test is for rapid identification of c.albicans. If the germ tube test is positive, further testing was not normally required.

DALMAU PLATE CULTURE

To study yeast morphology Dalmau plate culture techniques was followed.

Dalmau plate culture on Cornmeal tween 80 Agar

17 g corn meal agar in 1000 mL distilled water was dissolved and autoclaved. Sterile medium was poured into Petri dishes. All testing samples were freshly grown on YEPDA medium, and a tiny inoculum from the suspension cultures was streaked onto Corn Meal Agar (CMA) plates. Inoculated area was covered with the coverslips (22 × 22 mm). The growth plates were then incubated at 28°C, and colony characteristics were monitored every day for up to 72 hours.

Dalmau plate culture on Tobacco tween 80 Agar

This culture media was used for the differentiation among Candida albicans and Candida dubliniensis. Tobacco agar was prepared using the same technique as that stated by [25]. 10 g tobacco was dissolved in 1000 mL distilled water. After 15 minutes of boiling, the mixture was filtered through multiple layers of gauze. Agar (15 g) and Tween 80 (1%) was added, and mixture is boiled until solution is complete, after which it is autoclaved at 121°C for 15 min. Into petridishes, 20 mL of clean media were added (90 mm diameter). On tobacco agar plates, all test isolates that had previously been freshly grown on YEPD agar were streaked with a tiny amount of inoculum. The culture plates were cultured at 28°C, and colony features such surface characteristics (rough or smooth), hyphal fringe development at the periphery, and colony colour were assessed daily for up to 96 hours. Chlamydoconidia formation was also observed.

Biochemical Test for Characterization of Isolates.

Sugar Fermentation Test

Take 5mL YEPD broth and different sugars Glucose, Sucrose, Maltose, Trehalose, Galactose, Lactose and Glycerol (each contains 0.5%) along with pH indicator, bromothymol blue for acid detection. This indicator turns red at the pH 7.0 and yellow below pH 7.0. Durham tube was kept in inverted position in each tube for examined the gas production. Each tube was inoculated with test culture (10^6 CFU/mL) and incubated at 37°C for 24 h. The infected broth underwent a noticeable alteration as a result of the generation of acid, alkali, or gas. Purple colour showed no sugar fermentation. Yellow colour showed acid production only. Yellow colour and gas accumulated in Durham tube showed acid and gas production. Some yeast showed weak reaction after 48 h. In that case reaction was recorded as ± and incubated for further 24 h. Orange colour after 72 h of incubation was interpreted as negative reaction.

Screening for Fungal Immunity

Antifungal susceptibility testing was done by both Disc Diffusion (DD) and Microbroth Dilution (MBD) methods.

Antifungal Discs used

Fluconazole (FLZ) 25 µg, Amphotericin B (AMB) 100 units, Itraconazole (ITR) 10 µg, Clotrimazole (CTC) 10 µg and Ketoconazole (KET) 10 µg paper discs were used for this test. The interpretive breakpoints determined; CLSI M44-A and [26] were followed for all antifungal discs (Table 1).

Table 1. Interpretative zone sizes

Antifungal discs	**Drug content**	**MIC break points (mm) for following category**		
		Susceptible	***Dose dependent susceptible***	***Resistant***
Amphotericin B	100 units	≥19	12–18	≤12
Itraconazole	10 µg	≥19	12–18	≤12
Fluconazole	25 µg	≥19	15–18	≤14
Clotrimazole	10 µg	≥19	12–18	≤12
ketoconazole	10 µg	≥19	12–18	≤12

RESULTS AND DISCUSSION

Clinical Sample Collection

We have collected 8 oral cavity samples, 4 blood samples, 14 vaginal fluid samples and 16 urine samples from patients suspected of candidiasis.

ISOLATION OF YEASTS

The clinical samples obtained from the clinical lesions were aseptically inoculated on selective agar media including SDA and YEPDA with antibiotics and incubated at 37°C for 48 to 72 h to obtain discrete colonies and pure cultures.

IDENTIFICATION OF *CANDIDA* SPECIES

1 **By Microscopic Examination**
2. **Growth at 37°C**

Among all 24 yeasts isolates were grown successfully at 37°C and identifiable colonies were obtained after 48 h to 72 h of incubation (Table 2-4 and Figure 1-2).

Table 2. Cultural and biochemical Characteristics of 24 yeasts isolated from clinical samples.

Strain No.	**Growth at 37°C**	**Gram staining**	**Morphological characterization**			**Sugar fermentation**							**Interpretation**
			Pseudo hyphae	***chlamydospores***	***Germ tubes***	***Glucose***	***Maltose***	***Sucrose***	***Lactose***	***Galactose***	***Xylose***	***Trehalose***	
YOC1	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YOC2	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YOC3	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YOC4	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YOC5	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YOC6	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YV7	+	+	+	+	+	F	F	-	-	F	-	W	*C. albicans*
YV8	+	+	+	+	+	F	F	-	-	F	-	W	*C. albicans*

YU9	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YU10	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YU11	+	+	+	+	+	F	F	-	-	F	-	F	*C. albicans*
YU12	+	+	+	+	+	F	F	-	-	F	W	F	*C. albicans*
YU13	+	+	+	+	+	F	F	-	-	YU13	+	+	+
YU14	+	+	+	+	+	F	F	-	-	YU14	+	+	+
YU15	+	+	+	+	+	F	F	-	-	YU15	+	+	+
YU16	+	+	+	+	+	F	F	-	-	YU16	+	+	+
YV17	+	+	+	-F	-	F	F	F	-	YV17	+	+	+
YV18	+	+	+	-F	-	F	F	F	-	YV18	+	+	+
YU19	+	+	+	-F	-	F	F	F	-	YU19	+	+	+
YU20	+	+	+	-F	-	F	F	F	-	YU20	+	+	+
YU21	+	+	+	-F	-	F	F	F	-	YU21	+	+	+
YV22	+	+	-	-	-	F	-	-	-	YV22	+	+	-
YU23	+	+	-	-	-	F	-	-	-	YU23	+	+	-
YU24	+	+	-	-	-	F	-	-	-	YU24	+	+	-
+, positive reaction; -, negative reaction; *, strain variation; R, rare; F, the sugar is fermented (i. e., gas is produced); W, weak reaction; Nd, not detected.													

Table 3. Comparative distributions of all 24 yeast isolates from different clinical samples

Species	**Oral cavity (8)**	**Blood samples (4)**	**Vaginal fluid (14)**	**Urine samples (16)**
C. albicans (16)	6	0	2	8
	75%	0	14.2%	50%
C. tropicalis (5)	-	0	2	3
	-	0	14.2%	18.7%
C. glabrata (3)	-	0	1	2
	-	0	7.14%	12.5%
Total isolates (24)	6	0	5	13
	25%	0	35.7%	81.2%

Table 4. After 24 and 48 hours of incubation, in vitro antifungal against 24 yeast strains was assessed using the Disc Diffusion technique.

Strain No.	**Inhibition zone (mm) Zone size**					**Range**
	FLC	***ITR***	***CLC***	***KTC***	***AmB***	
YOC 1	18	20	19	25	22	6–32
	18	20	19	25	22	6–32
YOC2	16	19	17	21	19	6–32
	16	19	17	21	19	6–32
YOC3	18	19	17	31	22	6–32
	18	19	17	31	22	6–32
YOC4*	6	8	16	21	16	6–32
	6	8	16	21	16	6–32
YOC5*	7	8	18	20	14	6–32
	7	8	18	20	14	6–32
YOC6*	7	9	18	25	7	6–32
	7	9	18	25	7	6–32

YV7*	30	14	21	32	9	6–32

*= Drug resistant strain=fluconazole, ITR = Itraconazole, CLC = Clotrimazole, KTC = ketoconazole, AmB = Amphotericin B

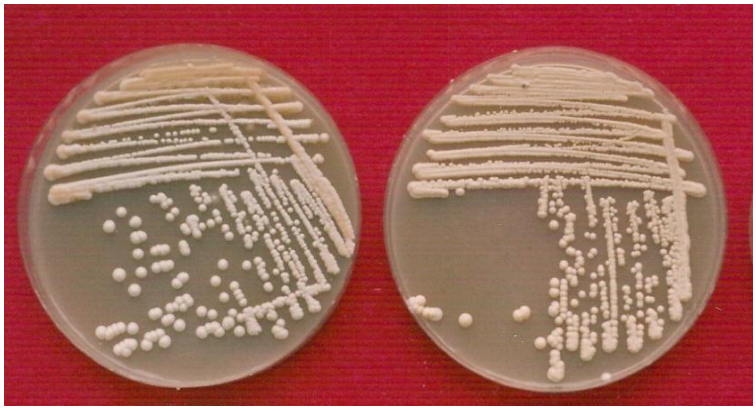

Figure 1. Growth of *Candida albicans* on YEPDA plates at 37°C.

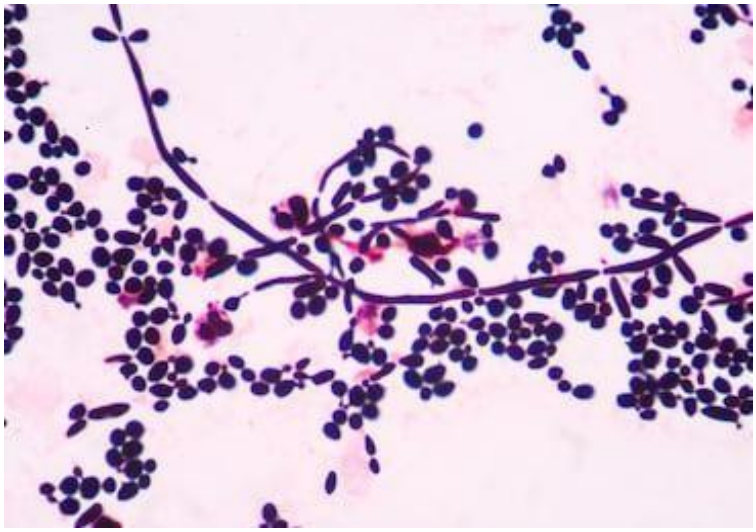

Figure 2. Microscopic examination of Candida by Gram's staining. Showing oval shaped budding yeast and pseudohyphae.

Germ Tube Test

Microscopic observation of the preparation revealed that the short hyphal initials produced by *C. albicans* were not constricted at the junction of the ballistoconidium and germ tube (Figures 3, 4).

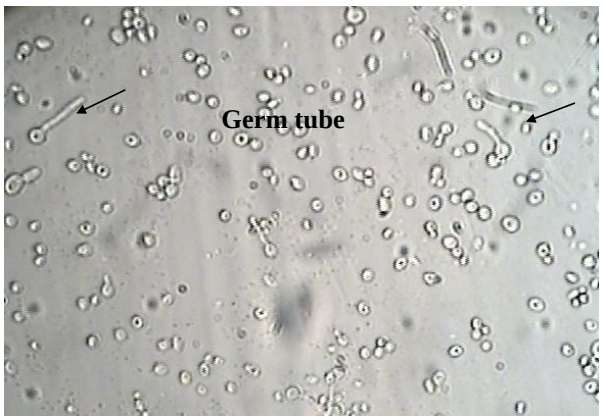

Figure 3. Identification of germ tube forms of *C. albicans* after 2 h of incubation in Human blood plasma (40X).

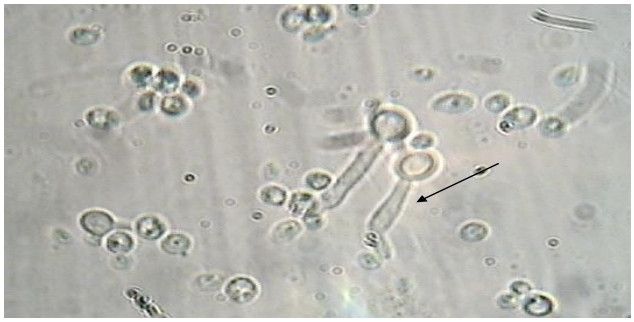

Figure 4. Germ tube formations for the identification of *C. albicans* after 2 h of incubation in Human blood plasma with 2% glucose (100X).

Morphological Studies

Candia albicans (Figures 5, 6 and 7). Growth of *C. tropicalis* was observed on CMA after 72 h of incubation. In case of *C.tropicalis* terminal vesicles (chlamydoconidia) are not produced (Figure 8)

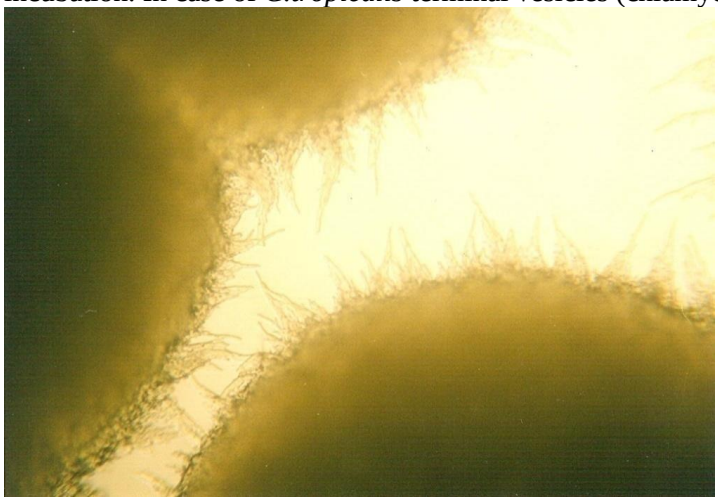

Figure 5. *C. albicans* formed rough and fringes colonies on tobacco agar plates incubated at 37ºC for 48 h (100X).

Figure 6. Growth of *C. albicans* on Tobacco agar after 72 h, pseudohyphae, blastoconidia, chlamydoconidia (terminally) 10X.

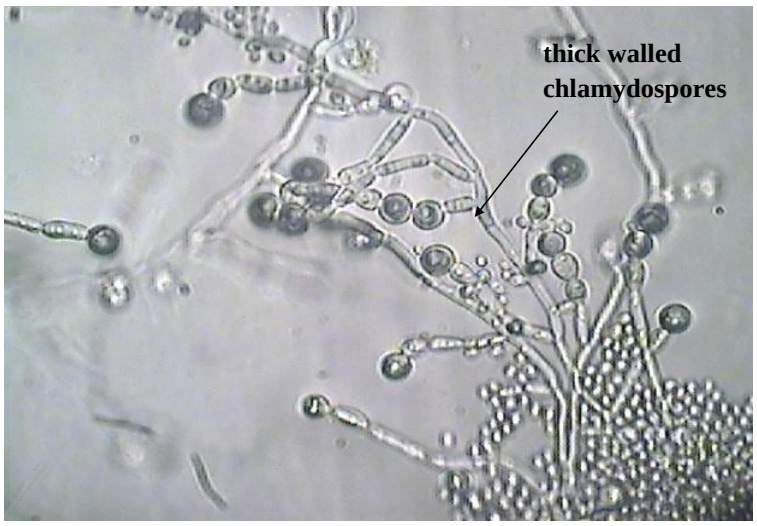

Figure 7. Growth of *C. albicans* on Tobacco agar after 72 h, pseudo hyphae, ballistoconidia, chlamydoconidium at terminal position of pseudo hyphae 40X.

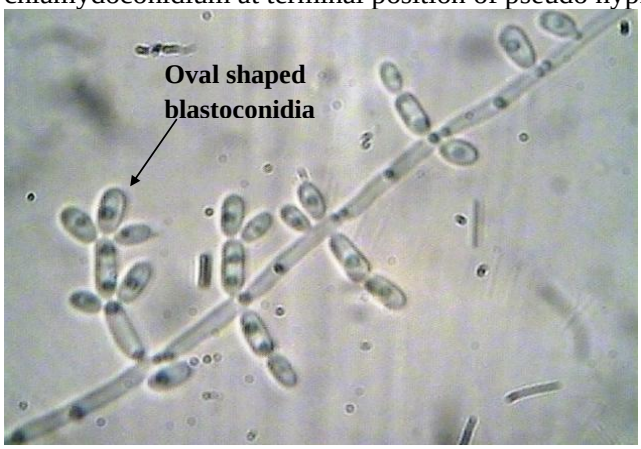

Figure 8. Growth of *Candida tropicalis* on Corn Meal Agar after 72 h, pseudo hyphae and ballistoconidia but no chlamydoconidium 40X.

Growth on HiCrome Agar

The properties of the colonies were clearly distinguished after 48 h of incubation:

- 12 of 16 *C. albicans* isolates grew as distinctive light green colonies on
- 5 C. albicans on HiCrome agar, tropicalis strains developed smooth, blue-violet colonies with a halo that spread into the surrounding agar (Figure 9).
- Three C. glabrata isolates generated pink, glossy, and pale-edged colonies.

For the purpose of identifying yeast species, this test is advised. Figure 10 shows sugar fermentation test for the identification *C. albicans.* Yellow color and gas accumulated in Durham tube shows acid and gas production.

Figure 9. Growth of yeast isolates on HI chrome *Candida* agar after 72 h of incubation at 37°C, Greenish blue colonies shows the presence of *Candida albicans.*

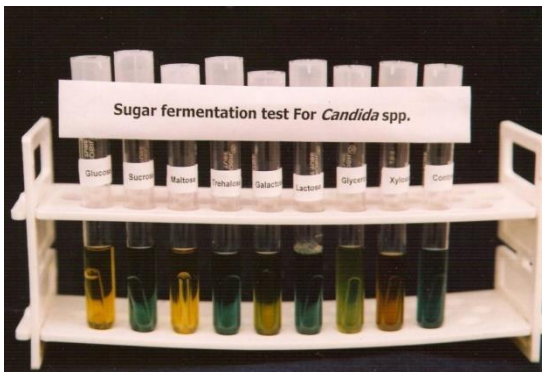

Figure 10. Sugar fermentation test for the identification of *Candida* species. Yellow colour indicates acid production. Bubble shows both acid and gas production.

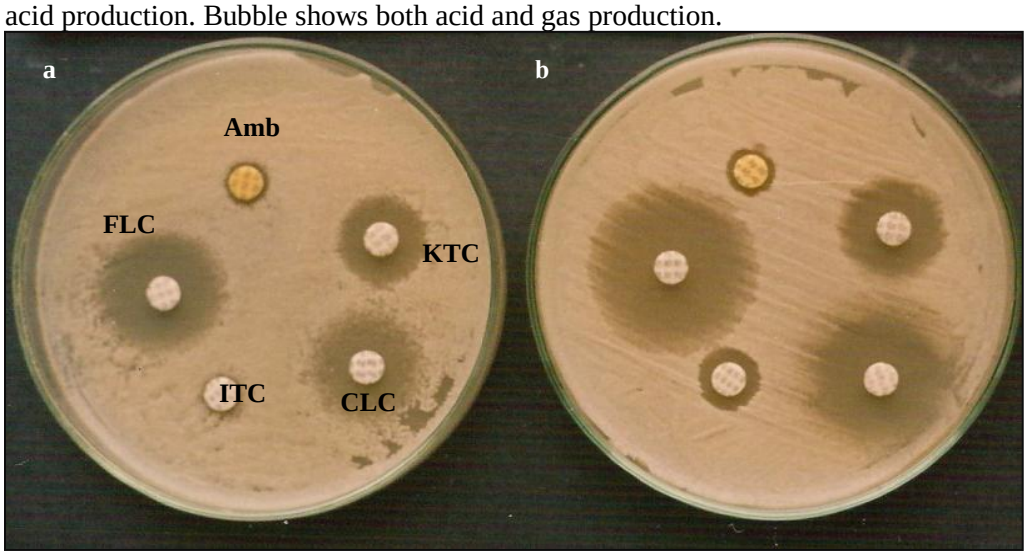

Figure 11. Antifungal susceptibility test by DD method using AmB 100units, KTC 10µg, CLC 10µg, FLC 25µg, ITC 10µg discs. (a) Strain number 20 (b) Strain number 7

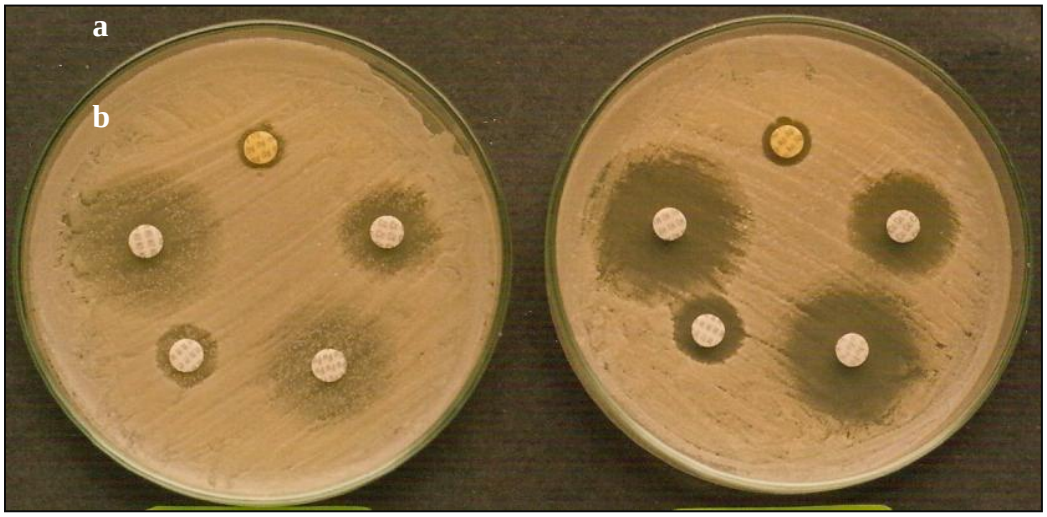

Figure 12. Antifungal susceptibility test by DD method by using AmB 100units, KTC 10µg, CLC 10µg, FLC 25µg, ITC 10µg discs. (a) Strain number 8 (b) Strain number 11

PREVALENCE OF *CANDIDA* CARRIAGE

Prevalence of *Candida* in Blood Aamples

In the present study we have evaluated 4 blood samples but no *Candida species* were found.

Prevalence of *Candida* in Urine Samples

The isolates 8 had C. albicans as the most prevalent species (50 percent), followed by C.tropicalis 3 (18.7%) and C. glabrata 2 (12.5%)

ANTIFUNGAL SUSCEPTIBILITY EVALUATION

We evaluated the antifungal sensitivity of 24 yeast isolates. This same DD test was used to analyze antifungal susceptibility in full compliance with CLSI guidelines (Table 5).

Fluconazole Resistance by Disc Diffusion Method

The supplemented MHA used for disc test supported the growth of all 24 yeasts isolates by using Fluconazole disc. (Figure 11-13 and Table 6).

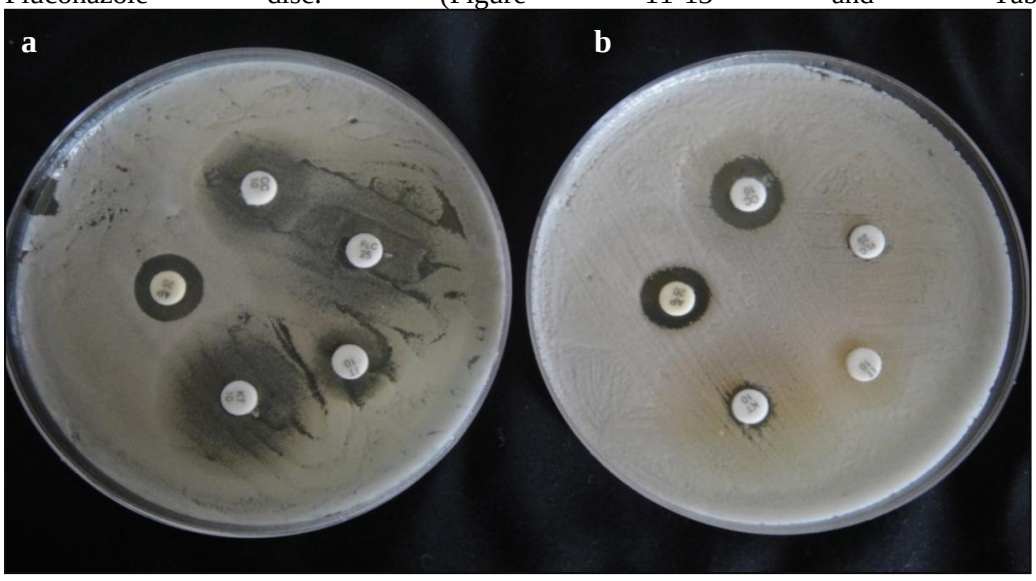

Figure 13. Antifungal susceptibility test by DD method for *Candida* species by using AmB 100 units, KTC 10µg, CLC 10µg, FLC 25µg, ITC 10µg discs. (a) Strain number 15 (b) Strain number 16

Table 5. (Continued). *In vitro* antifungal activity against 24 strains of yeast determined by disc diffusion method after 24 and 48 h of incubation.

Strain No.	Inhibition zone (mm) Zone size					Range
	FLC	***ITR***	***CLC***	***KTC***	***AmB***	
YU16*	7	6	8	15	14	6–32
	7	6	8	15	14	6–32
YV17	17	18	19	20	23	6–32
	17	18	19	20	23	6–32
YV18	18	20	18	20	20	6–32
	18	20	18	20	20	6–32
YU19	20	18	19	25	23	6–32
	20	18	19	25	23	6–32
YU20*	22	9	18	25	7	6–32
	22	9	18	25	7	6–32
YU21	20	18	19	25	22	6–32
	20	18	19	25	22	6–32
V22*	9	9	13	21	18	6–32
	9	9	13	21	18	6–32
YU23*	7	8	16	20	19	6–32
	7	8	16	20	19	6–32
YU24	16	14	16	21	20	6–32
	16	14	16	21	20	6–32

*= Drug resistant strain = fluconazole, ITR = Itraconazole, CLC = Clotrimazole, KTC = ketoconazole, AmB = Amphotericin B

Itraconazole Resistance by DD Method

Using Itraconazole disc (10 µg) susceptibility results were similar after 24 and 48 h of incubation (Figure 11-13 and Table 7).

Table 6. Comparison of Fluconazole resistance by specimen type and *Candida* spp.

Species		Specimen type				Total
		Oral cavity	***Blood isolates***	***Vaginal fluid***	***Urine isolates***	
C. albicans	N	6	-	2	8	16
	NR	3	-	0	1	4
	PR	50%	-	0	12.5%	25%
C. tropicalis	N	-	-	2	3	5
	NR	-	-	0	0	0
	PR	-	-	0	0	0
C. glabrata	N	-	-	1	2	3
	NR	-	-	1	1	2
	PR	-	-	100%	50%	66.6%
Total	N	6	-	5	13	24
	NR	3	-	1	2	6
	PR	50%	-	20%	15.38%	25%

N = number of isolates; NR = number of isolates resistant; PR = Percentage of isolates resistant

Clotrimazole Resistance by DD Method

Using clotrimazole disc (10 µg) susceptibility results were similar after 24 and 48 h of incubation (Figure 11-13 and Table 8).

Table 7. Comparison of Itraconazole resistance by specimen type and *Candida* spp.

Species		Specimen type				Total
		Oral cavity	***Blood isolates***	***Vaginal fluid***	***Urine isolates***	
C. albicans	N	6	-	2	8	16
	NR	3	-	0	2	5
	PR	50%	-	0	25%	31.25%
C. tropicalis	N	-	-	2	3	5
	NR	-	-	0	1	1
	PR	-	-	0	33.3%	20%
C. glabrata	N	-	-	1	2	3
	NR	-	-	1	1	2
	PR	-	-	100%	50%	66.6%
Total	N	6	-	5	13	24
	NR	3	-	1	4	8
	PR	50%	-	20%	30.76%	33.3%

N = number of isolates; NR = number of isolates resistant; PR = Percentage of isolates resistant

Ketoconazole Resistance by DD Method

Using ketoconazole disc (10 µg) susceptibility results were similar after 24 and 48 h of incubation (Figure 11-13 and Table 9).

Table 8. Comparison of Clotrimazole resistance by specimen type and *Candida* spp.

Species		Specimen type				Total
		Oral cavity	***Blood isolates***	***Vaginal fluid***	***Urine isolates***	
C. albicans	N	6	-	2	8	16
	NR	0	-	0	1	1
	PR	0	-	0	12.5%	6.25%
C. tropicalis	N	-	-	2	3	5
	NR	-	-	0	0	0
	PR	-	-	0	0	0
C. glabrata	N	-	-	1	2	3
	NR	-	-	0	0	0
	PR	-	-	0	0	0
Total	N	6	-	5	13	24
	NR	0	-	0	1	1
	PR	0	-	0	7.69%	4.16%

N = number of isolates; NR = number of isolates resistant; PR = Percentage of isolates resistant

Amphotericin B Resistance by DD Method

Using amphotericin B disc (10µg) for disc diffusion test susceptibility results were similar after 24 and 48 h of incubation (Figure 11-13 and Table 10).

Table 9. Comparison of Ketoconazole resistance by specimen type and *Candida* spp.

Species		Specimen type				Total
		Oral cavity	**Blood isolates**	**Vaginal fluid**	**Urine isolates**	
C. albicans	N	6	-	2	8	16
	NR	0	-	0	0	0

	PR	0	-	0	0	0
C. tropicalis	N	-	-	2	3	5
	NR	-	-	0	0	0
	PR	-	-	0	0	0
C. glabrata	N	-	-	1	2	3
	NR	-	-	0	0	0
	PR	-	-	0	0	0
Total	N	6	-	5	13	24
	NR	0	-	0	0	0
	PR	0	-	0	0	0

N = number of isolates; NR = number of isolates resistant; PR = Percentage of isolates resistant

Table 10. Comparison of Amphotericin B resistance by specimen type and *Candida* spp.

Species		**Specimen type**				**Total**
		Oral cavity	***Blood isolates***	***Vaginal fluid***	***Urine isolates***	
C. albicans	N	6	-	2	8	16
	NR	1	-	2	1	4
	PR	16.6%	-	100%	12.5%	25%
C. tropicalis	N	-	-	2	3	5
	NR	-	-	0	1	1
	PR	-	-	0	33.3%	20%
C. glabrata	N	-	-	1	2	3
	NR	-	-	0	0	0
	PR	-	-	0	0	0
Total	N	6	-	5	13	24
	NR	1	-	2	2	5
	PR	16.6%	-	40%	15.38%	20.83%

N = number of isolates; NR = number of isolates resistant; PR = Percentage of isolates resistant

CONCLUSION

In this study, we have concluded that prevalence candidiasis infection is increasing in Dehradun city. *Candida* species isolates showing 33.3%, 25.0% resistance against itraconazole and fluconazole respectively. Systemic infection due to yeast and resistance to antifungal is on rise in Indian population. Identification of germ tube forms

REFERENCES

1. Lehrer RI, Cline MJ. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to Candida infection. The Journal of clinical investigation. 1969; 48 (8): 1478–88.
2. Gruby D. Recherches anatomiques sur une plante cryptogame qui constitue le vrai muguet des enfants. CR Acad Sci (Paris). 1842; 14: 634–6.
3. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. Reviews of infectious diseases. 1990; 12 (2): 308–29..
4. Benson, H.J. Microbiological applications: A laboratory manual in general Microbiology. McGraw-Hill Education; 8th edition (16 August 2001).
5. Ashman RB, Papadimitriou JM. What's new in the mechanisms of host resistance to Candida albicans infection? Pathol Res Pract. 1990; 186 (4):527–34.
6. Parisa Badiee, et al. Antifungal susceptibility testing of Candida species isolated from the immunocompromised patients admitted to ten university hospitals in Iran: comparison of colonizing and infecting isolates. BMC Infect Dis .2017 Nov 21;17(1):727.
7. Paldrok H. Medical mycology in scandinavia and Finland during a century, from 1841 to 1940.

Mycopathologia et mycologia applicata. 1960; 12 (4): 289–348.
8. Barry AL, Pfaller MA, Brown SD et al., Rinaldi MG. Quality control limits for broth microdilution susceptibility tests of ten antifungal agents. Journal of Clinical Microbiology. 2000; 38 (9): 3457–9.
9. Masson PL, Heremans JF. Lactoferrin in milk from different species. Comparative Biochemistry and Physiology. 1971 (1): 119–29.
10. Brown LR, Dreizen S, Handler S, Johnston DA. Effect of radiation-induced xerostomia on human oral microflora. Journal of dental research. 1975; 54 (4): 740–50.
11. Nikawa H, Samaranayake LP, Tenovuo J, Pang KM, Hamada T. The fungicidal effect of human lactoferrin on Candida albicans and Candida krusei. Archives of oral biology. 1993; 38 (12): 1057–63.
12. Samaranayake LP, Raeside JM, MacFarlane TW. Factors affecting the phospholipase activity of Candida species in vitro. Sabouraudia: Journal of Medical and Veterinary Mycology. 1984; 22 (3): 201–7.
13. Kelly SL, Lamb DC, Baldwin BC,et.el,. Characterization of Saccharomyces cerevisiae CYP61, sterol Δ22-desaturase, and inhibition by azole antifungal agents. Journal of Biological Chemistry. 1997; 272 (15): 9986–8.
14. Antley PP, Hazen KC. Role of yeast cell growth temperature on Candida albicans virulence in mice. Infection and immunity. 1988;56 (11): 2884–90.
15. Grappel SF, Calderone RA. Effect of antibodies on the respiration and morphology of Candida albicans. Sabouraudia: Journal of Medical and Veterinary Mycology. 1976; 14 (1): 51–60.
16. Torheim BJ. Immunochemical investigations in Geotrichum and certain related fungi: I. Introduction and description of the strains. Sabouraudia: Journal of Medical and Veterinary Mycology. 1963; 2 (3): 146–54.
17. Borg M, Rüchel R. Expression of extracellular acid proteinase by proteolytic Candida spp. during experimental infection of oral mucosa. Infection and Immunity. 1988r; 56 (3): 626–31.
18. Bhatnagar, B. and Garg, A.P, Qualitative and Quantitative analysis of microfungi colonizing leaf-litter of *Oryza sativa L. J. Indian Bot. Soc.1990;* 69: 81–87.
19. Allen CM, Beck FM. Strain-related differences in pathogenicity of Candida albicans for oral mucosa. Journal of Infectious Diseases. 1983; 147 (6): 1036–40.
20. Capoor MR, Nair D, Deb M, Verma PK, Srivastava L, Aggarwal P. Emergence of non-albicans Candida species and antifungal resistance in a tertiary care hospital. Japanese journal of infectious diseases. 2005; 58 (6): 344.
21. Goldstein AL, McCusker JH. Development of Saccharomyces cerevisiae as a model pathogen. A system for the genetic identification of gene products required for survival in the mammalian host environment. Genetics. 2001; 159 (2): 499–513.
22. Imam N, Carpenter CC, Mayer KH, et al. Hierarchical pattern of mucosal candida infections in HIV-seropositive women. Am J Med. 1990; 89 (2):142–6.
23. Scully C, Ei-Kabir M, Samaranayake LP. Candida and oral candidosis: a review. Critical Reviews in Oral Biology & Medicine. 1994; 5 (2): 125–57.
24. Marr, K.A.; Lyons, C.N.; Rustad, T.R.. Rapid, transient fluconazole resistance in *Candida albicans* is associated with increased mRNA levels of CDR. *Antimicrob. Agents Chemother. 1998;* 42 (10): 2584–2589.

