E-ISSN 2250-0944

ISSN 2250-1150

doi: 10.48047/ijprt/15.02.156

PREVALENCE OF CANDIDA INFECTIONS AND THEIR RESISTANCE PROFILE AMONG PATIENTS AT TERTIARY CARE HOSPITALS, NORTH-INDIA

Ashu Rajput[1], Dr. Madhurendra Rajput[2], Chaman Parveen[3], Dr. Vishal Jain[4]

[1]Ph.D scholars (Medical Microbiology), [2]Professor, Department of Microbiology, Malwanchal University, Indore Madhya Pradesh, India, Assistant Professor, [4]Department of Surgery, ASMC, Lalitpur

Corresponding author

Ashu Rajput

[1]Ph.D scholars (Medical Microbiology), Department of Microbiology, Malwanchal University, Indore Madhya Pradesh, India,

Email:ashurajput2437@gmail.com

ABSTRACT

Introduction- The term mycology is derived from the Greek word 'mykes', a direct counterpart of the Latin word 'fungus', which is in turn thought to be a modification of the Greek word 'sponges', from which our word "sponge" is derived. In last 30 years there has been a significant increase in the incidence of fungal infections in humans.Such infections may either be superficial, affecting the skin, hair, nails and mucosal membranes, or systemic, involving major body organs. A number of factors have been implicated in this increased occurrence of fungal disease, such as immunosuppressive therapies, invasive surgical procedures and use of broad-spectrum antibiotics are significant. **Aim** - To detect the prevalence of *Candida* infection and their resistance pattern among patients. **Objectives** - To detect clinical pattern of *Candida* infection by isolating the *Candida* on Sabouraud's Dextrose Agar (SDA).To isolate the *Candida*, identify them upto species level. **Material& Method**- This prospective cross-sectional study was conducted in the Mycology Laboratory, Department of Microbiology.Prior informed consent and demographic details of the study subjects were collected before processing each sample for fungal profiling. Patients who withdrew consent at any stage of the study were excluded. This study was performed with routine samples from different inpatient departments received in the Bacteriology laboratory for bacterial culture and sensitivity. A prior consent was taken from each patient.**Result-** This study was carried in the Department of Microbiology. A total of **2658** specimens were tested, out of which, 111 non-repetitive specimens received from various wards, and intensive care units were included in this study, with the prevalence of 4.17%. Male patients were 57 (51.35%) and female patients were 54 (48.64%) Overall, 111 isolates of *Candida* species were recovered, including *C. albicans* and non-*albicans Candida* species. Among the recovered isolates, the NAC were higher in number with 62 isolates (55.8%) than *C. albicans*. The highest proportion of isolates (species-wise) was that of *C. albicans* with 49 isolates (44.1%). *C. tropicalis* held the with 25 isolates (22.5%), followed by 16 isolates (14.4%) and 14 isolates (12.6%) of *C. glabrata* and *C. krusei*, respectively, **Conclusion-** The *Candida* species are opportunistic pathogenic organisms, but they may also cause superficial and systemic infections in the presence of predisposing factors. The presence of a central venous catheter, use of broad spectrum antibiotics, prolonged stay in intensive care units, mechanical ventilation, parenteral nutrition, dialysis, immunodeficiency, and diabetes mellitus compose the predisposing factors for candidiasis. Infections caused by Candida species are significantly increasing today. **Keyword- prevalence**, candida, Resistant profile,

INTRODUCTION

The term **mycology** is derived from the Greek word '**mykes'**, a direct counterpart of the Latin word 'fungus', which is in turn thought to be a modification of the Greek word 'sponges', from which our word "sponge" is derived.[1]

In last 30 years there has been a significant increase in the incidence of fungal infections in humans.[2] Such infections may either be superficial, affecting the skin, hair, nails and mucosal membranes, or systemic, involving major body organs.[3] A number of factors have been implicated in this increased occurrence of fungal disease, such as immunosuppressive therapies, invasive surgical procedures and use of broad-spectrum antibiotics are significant. [4]

The history of candidiasis is very old as the disease was described in ancient times and gradually its etiological agents, diagnostic procedures and therapeutic modalities came into existence. The first known description of candidal infections as oral thrush in patients with underlying diseases may be found in Hippocrates' *'Epidemics'* from the fourth century BC. Rosen von Rosenstein and Underwood identified candidal infections in pediatric patients and made the first description of thrush in modern medicine.

There are two popular terms, **candidosis** and **candidiasis**, which are frequently used and both are correct, however, candidosis is often preferred in some of the European countries like United Kingdom, France and Italy as well as Canada giving disease name on the basis of pattern followed in other fungal diseases by putting ***'osis'*** as suffix.

Candida albicans is the pathogen identifed in most patients with candidiasis, but other possible pathogens also include non-albicans Candida species (NACS) as *Candida glabrata, Candida parapsilosis, Candida krusei, Candida tropicalis, Candida dubliniensis, Candida lusitaniae,* and *Candida auris*.[5]

MATERIAL AND METHOD

This prospective cross-sectional study was conducted in the Mycology Laboratory, Department of Microbiology.

Prior informed consent and demographic details of the study subjects were collected before processing each sample for fungal profiling. Patients who withdrew consent at any stage of the study were excluded.

Specimens

This study was performed with routine samples from different inpatient departments received in the Bacteriology laboratory for bacterial culture and sensitivity. A prior consent was taken from each patient.

Isolation

The preliminary diagnoses of specimens were performed by wet mount, Gram stain, culture on Sabouraud dextrose agar (SDA) and urea hydrolysis test. [6]

Identification

Any visible growth seen on SDA slope was processed for identifcation of the species. From an isolated colony, macroscopic examination, Gram staining, germ tube test. The yeasty, pasty and creamy colony that showed Gram positive budding yeast cells with pseudohyphae on microscopic examination and negative urea hydrolysis test were further processed for *Candida* speciation on CHROM agar.[7]

Germ Tube Production Test (GTT) (Reynolds Braude Phenomenon)

This method is used for presumptive identification of *C. dubliniensis* and *C. albicans*. Long tube-like projections arising directly from the mother cells and no constriction at the point of origin is identified as germ tube. [8]

Cornmeal Agar (CMA) with Tween 80 (Dalmau plate culture)

Growth of *Candida* on CMA reveals distinct morphology regarding chlamydospores, blastospores and hyphae development. CMA containing 1% Tween 80 was prepared as per manufacturer's (HiMedia) instructions. Each *Candida* spp. was inoculated on cornmeal agar as three parallel streaks approximately 3 mm apart, covered with sterile coverslip and incubated in wet chamber for 48-72 hours at 25°C. [9]

Growth on CHROM agar

CHROM agar is differential medium which has been used for simultaneous isolation and identification of different *Candida* spp. Identification of *Candida* spp. was done on the basis of colour formation and colony morphology.[10]

Sugar Fermentation Test[46]

- Liquid fermentation media was prepared containing the six filter sterilized sugars Glucose, Sucrose, Lactose, Maltose, Galactose and Trehalose.
- Inoculum was prepared from growth of *Candida* isolates from sugar free medium and inoculated with 0.1ml in each sugar tube tested.
- Tubes were incubated for 7 days at 25°C and observed daily. **[11]**

Sugar Assimilation Test (Auxanographic technique)

Disc Impregnation Pour plate method was used to perform the Sugar assimilation test. The steps are as follows:

- *Candida* culture suspension was prepared in 2 ml of sterile yeast nitrogen base (YNB) (HiMedia), and

added to the cooled (45°C) 18 ml molten YNB agar.

- After proper mixing, the entire content was poured into sterile petri plate and allowed to set at room temperature. [11]

RESULTS

A total of **2658** specimens were tested, out of which, 111 non-repetitive specimens received from various wards, and intensive care units were included in this study, with the prevalence of 4.17%.

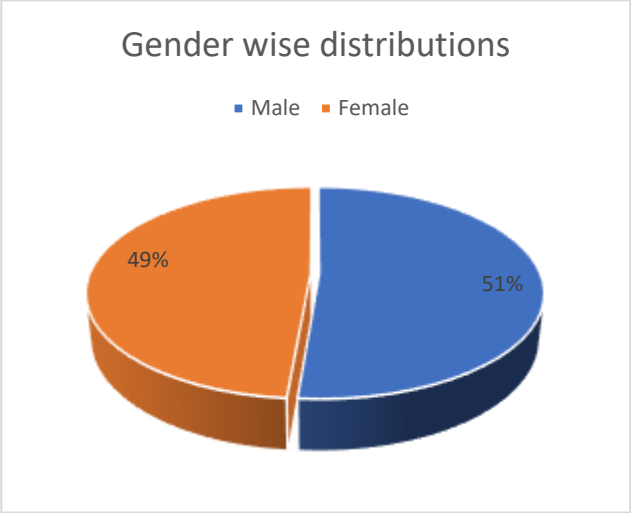
Gender Wise Distribution

Male patients were 57 (51.35%) and female patients were 54 (48.64%) as demonstrated in **Table 1** and **Pie Chart 1.** The male-female ratio was 1.05: 1.

Table 1: Gender-based distribution of patients

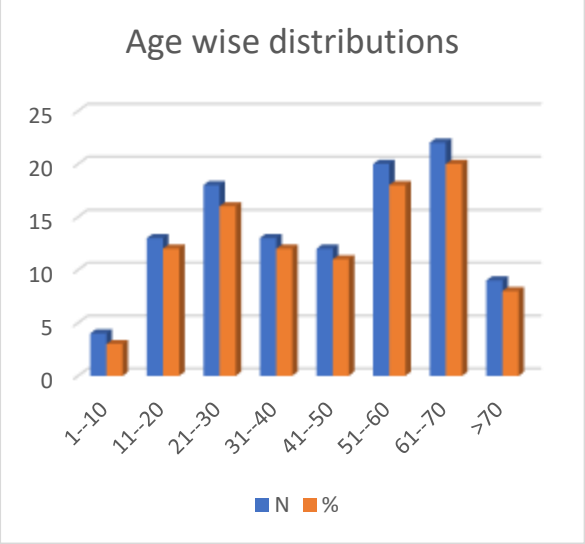
Gender	N	%
Male	57	51.35
Female	54	48.64

Pie Chart 1: Gender-based distribution of patients



Age group wise distribution

The patients aged between 3 years and 85 years with the mean age of 45.79 years. They were divided into several age groups as demonstrated in **Bar Chart-2**.



Bar Chart 2: Frequency polygon of age-wise distribution of patients

Specimen source distribution

The patients were distributed throughout the hospital facilities and units, and have thus

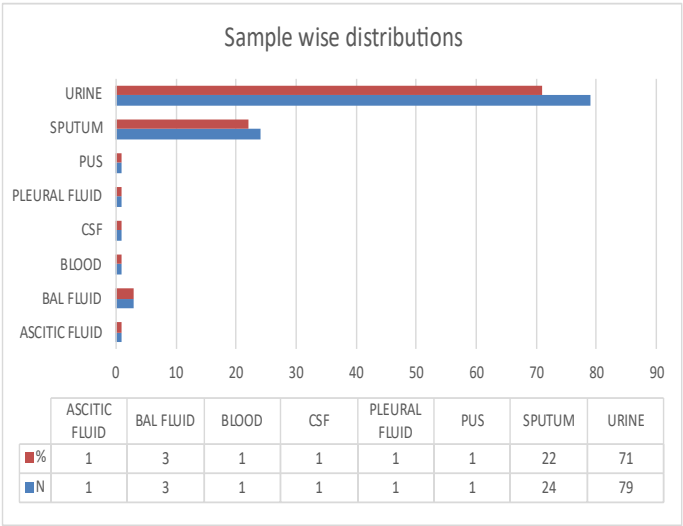
been divided into 10 sections. Majority of the isolates of *Candida* were recovered from Medicine intensive care (MICU), Respiratory Medicine Ward (RMW) and Out Patient Department (OPD), contributing to 39 isolates (39.13%), 22 isolates (19.81%) and 19 isolates (17.11%) respectively. It was followed by Male Medicine Ward (MMW), Paediatric Ward, Female Medicine Ward contributing 13 (11.71%), 7 (6.30%), 6 (5.40%) respectively. Distribution of specimen source can be seen in **Table 3**

Table 5.3: Patient distribution with respect to wards

WARDS	**N**	**%**
COVID WARD	1	1
EMERGENCY DEPARTMENT (EMG)	1	1
EAR NOSE & THROAT (ENT)	2	2
FEMALE MEDICINE WARD (FMW)	6	5
MEDICINE ICU (MICU)	39	35
MALE MEDICINE WARD (MMW)	13	12
OUT PATIENT DEPARTMENT (OPD)	19	17
PAEDIATRIC WARD	7	6
PAEDIATRIC ICU	1	1
RMW (RESPIRATORY MEDICINE WARD)	22	20
Total	111	100

Sample wise distribution

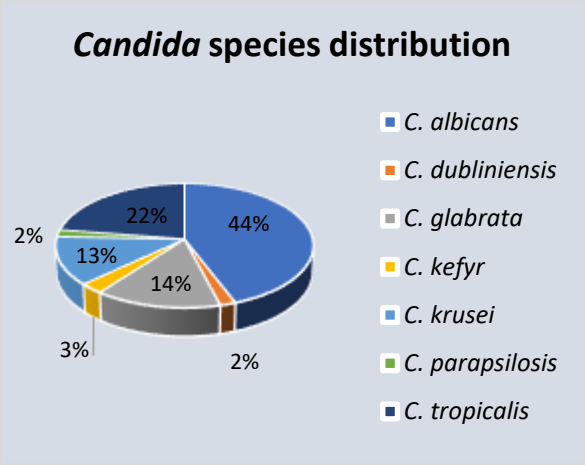
Out of the total 111 isolates, 79 isolates (71.1%) were recovered from urine, followed by 24 isolates (21.6%) from sputum and 3 isolates from Broncho alveolar lavage fluid (BAL Fluid) (2.7%), respectively. Though smaller in number, but isolates of *Candida* species were recovered from other body specimens too, including 1isolate from Ascitic fluid, 1 from Pleural fluid, 1 isolate from Blood, 1 isolate from CSF and 1 isolate from pus. Distribution of isolates according to specimen has been demonstrated in **Bar Chart - 4**



Bar Chart.4: Sample wise distribution

Candida species distribution

Overall, 111 isolates of *Candida* species were recovered, including *C. albicans* and non-*albicans Candida* species. Among the recovered isolates, the NAC were higher in number with 62 isolates (55.8%) than *C. albicans*. The highest proportion of isolates (species-wise) was that of *C. albicans* with 49 isolates (44.1%). *C. tropicalis* held the with 25 isolates (22.5%), followed by 16 isolates (14.4%) and 14 isolates (12.6%) of *C. glabrata* and *C. krusei*, respectively, as demonstrated in **Pie Chart 5** along with the other recovered isolates.



Pie Chart 5: Distribution of recovered *Candida* isolates

DISCUSSION

Candida species are the most common cause of invasive fungal infections in humans, producing infections that range from non–life-threatening mucocutaneous disorders to invasive disease that can involve any organ.[90] *Candida* species are among the most common causes of nosocomial bloodstream infections and of invasive infections in intensive care units (ICUs). Timely antifungal therapy and source control are crucial determinants of survival in patients with invasive candidiasis.[12]

Infections of the mucous membranes are associated with defects in cellular immunity such as the depletion of CD4-positive T-helper cells in patients with HIV infection, after haematopoietic stem cell transplantation, in patients treated with steroids and antineoplastic agents (e.g. fludarabine), in graft versus-host disease (GvHD) or after radiation therapy. Other predisposing factors include diabetes mellitus, therapy with antibacterial agents or local factors such as the use of a dental prosthesis.[13]

In line with the increasing incidence of invasive fungal infections and the growing number of available antifungal agents, and due to increase in number of antifungal resistant strains, there is a need for accurate, reproducible and clinically relevant susceptibility testing of fungal isolates to enable physicians to make informed decisions regarding treatment. The Clinical and

Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have both developed standard susceptibility testing methods for Candida species based on broth microdilution.[16]

A prospective study was carried out with one hundred eleven (111) clinical specimens, with suspected fungal infections from January 2022 January 2024 including 6 months of data analysis, North India, a tertiary care hospital.

In the present study, *Candida* prevalence was realized to be 4.17%. A recent study conducted by **Jayant S** *et al.,* (2021) stated the prevalence of 0.86%[14]. While the study conducted by **Sanguinetti M** *et al.,* (2015) prevalence was 0.69%[15]. In another study conducted by **Al Halteet S**, et al., (2020) prevalence of *Candida* was 15.6%.[16]

In the present study, it was found that *Candida* infections occurred in both sexes and at all ages with a male predominance (51.35%). In a study conducted by **Yang Z** *et al.,* (2022) have reported male predominance of 61.65%.[17] High male predominance in the present study highlights the fact that males are exposed to a higher risk of acquiring *Candida* infections as compared to females because of their jobs and habits make them more migratory, involved in outdoor activities, and labour work as well. The adult age group is the working productive age group of a nation. Due to active reproductive age, females are also prone to acquire an infection. As India is a tropical country, high temperature augments *Candida* infection.

We found the mean age of 45.79 years in the present study, while in the study by **Vaezi A** *et al.,* (2017), the mean age was higher 46.8 years, this is in concordance with our study.[18]

In the present study, the highest frequency of *Candida* isolation was observed in the age group of 61-70 years with 20% isolation. In a study conducted by **Da Costa VG** *et al.,* (2014) frequency of isolates of in the age group of 61 to 70 years is 25.8%.[19]

In the present study, the highest number of samples received were urinary samples (71%), followed by sputum samples (22%) and bronchoalveolar lavage fluid (3%).In a study conducted by **Pote ST** *et al.,* (2020) highest number of *Candida* isolates were found in urine (43.75%), followed by sputum samples (18.75%).[10]

In the Present study, maximum number of isolates belong to *Candida albicans* (44.1%), followed by *Candida tropicalis* (22.5%) and *Candida glabrata* (14.4%) and *Candida krusei* (12.6%) respectively. In a similar study, done by **Verma R** *et al.,* (2021) *Candida albicans* was the major species accounting for 37.95% of total isolates followed by *Candida tropicalis* (29.40%),

Candida glabrata (11.68%) and *Candida parapsilosis* (8.36%).[11]

CONCLUSION

The *Candida* species are opportunistic pathogenic organisms, but they may also cause superficial and systemic infections in the presence of predisposing factors. The presence of a central venous catheter, use of broad spectrum antibiotics, prolonged stay in intensive care units, mechanical ventilation, parenteral nutrition, dialysis, immunodeficiency, and diabetes mellitus compose the predisposing factors for candidiasis. Infections caused by Candida species are significantly increasing today. *Candida albicans (C.albicans)* is the most common species, but the burden of non *albicans Candida* species is increasing.

Our study was conducted on 111 candida isolates mainly from rural population. Study was carried out in a span from January 2022 January 2024 including 6 months of data analysis.

In our study the prevalence of *Candida* was 4.17%. Male patients were 57 (51.35%) and female patients were 54 (48.64%).

Maximum no of patients were in the age group of 61-70yrs, minimum were in 1-10yrs of age group. Mean age group of study subjects was 45.79 years.

Majority of the isolates of *Candida* were recovered from Medicine intensive care (MICU), Respiratory Medicine Ward (RMW) and Out Patient Department (OPD), contributing to 39 isolates (39.13%), 22 isolates (19.81%) and 19 isolates (17.11%) respectively.

Out of the total 111 isolates, 79 isolates (71.1%) were recovered from urine, followed by 24 isolates (21.6%) from sputum and 3 isolates from Broncho alveolar lavage fluid (BAL Fluid) (2.7%), respectively. Single *Candida* isolate was recovered from each of the other body fluid specimens too (Ascitic fluid, Pleural fluid, Blood, CSF and pus).

Among the recovered isolates, highest proportion of isolates (species-wise) was that of *C. albicans* with 49 isolates (44.1%). *C. tropicalis* held the with 25 isolates (22.5%), followed by 16 isolates (14.4%) and 14 isolates (12.6%) of *C. glabrata* and *C. krusei*, respectively

REFERENCES

1) Chander J. Textbook of medical mycology: Introduction. 4th edition. NewDelhi: Jaypee publishing; 2018.
2) Lass-Flörl C. The changing face of epidemiology of invasive fungal disease in Europe. Mycoses. 2009 May;52(3):197-205.
3) Rüping MJ, Vehreschild JJ, Cornely OA. Patients at high risk of invasive

fungal infections. Drugs. 2008 Oct;68(14):1941-62.

4) Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. FEMS microbiology reviews. 2012 Mar 1;36(2):288-305.
5) Ré AC, Martins JF, Cunha-Filho M, Gelfuso GM, Aires CP, Gratieri T. New perspectives on the topical management of recurrent candidiasis. Drug Delivery and Translational Research. 2021 Aug;11(4):1568-85.
6) Saigal S, Bhargava A, Mehra SK, Dakwala F. Identification of *Candida albicans* by using different culture medias and its association in potentially malignant and malignant lesions. Contemporary clinical dentistry. 2011 Jul;2(3):188.
7) Gautam G, Rawat D, Kaur R, Nathani M. Candidemia: Changing dynamics from a tertiary care hospital in North India. *Curr Med Mycol*. 2022 Mar;8(1):20–25. doi:10.18502/cmm.8.1.9210. PMID: 36340431; PMCID: PMC9548083.
8) Walker L, Huppert M. Corn Meal-Tween Agar: an Improved Medium for the Identification of *Candida albicans*. American journal of clinical pathology. 1960;33(2):190-94.
9) Golia, S., Reddy., K.M., Sujatha, K., & Hittinahalli, V. (2013). Speciation of *Candida* using chromogenic and cornmeal agar with determination of fluconazole sensitivity. Verma R, Pradhan D, Hasan Z, Singh H, Jain AK, Khan LA. A systematic review on distribution and antifungal resistance pattern of *Candida* species in the Indian population. Medical mycology. 2021 Dec;59(12):1145-65.
10) Odds FC, Bernaerts RI. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida species*. Journal of clinical microbiology. 1994 Aug;32(8):1923-9.
11) Jeddy N, Ranganathan K, Devi U, Joshua E. A study of antifungal drug sensitivity of *Candida* isolated from human immunodeficiency virus infected patients in Chennai, South India. Journal of oral and maxillofacial pathology: JOMFP. 2011 May;15(2):182.
12) Ruhnke M, Rickerts V, Cornely OA, Buchheidt D, Glöckner A, Heinz W, Höhl R, Horre R, Karthaus M, Kujath P, Willinger B. German Speaking Mycological Society, Paul-Ehrlich-Society for Chemotherapy. Diagnosis and therapy of *Candida* infections: joint

recommendations of the German Speaking Mycological Society and the Paul-Ehrlich-Society for Chemotherapy. Mycoses. 2011 Jul;54(4):279-310

13) Sanguinetti M, Posteraro B, Lass-Flörl C. Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. Mycoses. 2015 Jun;58:2-13.

14) Jayant S, Patel K, Priya P, Verma AN, Singh B, Dahariya R. Prevalence of *Candida* infection in Covid-19 pandemic: A study from a tertiary care center in Central India. Asian Journal of Medical Sciences. 2021 Oct 1;12(10):3-7.

15) Sanguinetti M, Posteraro B, Lass-Flörl C. Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. Mycoses. 2015 Jun;58:2-13.

16) Al Halteet S, Abdel-Hadi A, Hassan M, Awad M. Prevalence and antifungal susceptibility profile of clinically relevant candida species in postmenopausal women with diabetes. BioMed Research International. 2020 Nov 26;2020.

17) Yang Z, Zhang F, Li D, Wang S, Pang Z, Chen L, Li R, Shi D. Correlation Between Drug Resistance and Virulence of *Candida* Isolates from Patients with Candidiasis. Infection and Drug Resistance. 2022 Dec 31:7459-73.

18) Vaezi A, Fakhim H, Khodavaisy S, Alizadeh A, Nazeri M, Soleimani A, Boekhout T, Badali H. Epidemiological and mycological characteristics of candidemia in Iran: a systematic review and meta-analysis. Journal de mycologie medicale. 2017 Jun 1;27(2):146-52.

19) Da Costa VG, Quesada RM, Abe AT, Furlaneto-Maia L, Furlaneto MC. Nosocomial bloodstream *Candida* infections in a tertiary-care hospital in South Brazil: a 4-year survey. Mycopathologia. 2014 Oct;178(3):243-50.

20) Pote ST, Sonawane MS, Rahi P, Shah SR, Shouche YS, Patole MS, Thakar MR, Sharma R. Distribution of pathogenic yeasts in different clinical samples: their identification, antifungal susceptibility pattern, and cell invasion assays. Infection and drug resistance. 2020;13:1133.

21) Verma R, Pradhan D, Hasan Z, Singh H, Jain AK, Khan LA. A systematic review on distribution and antifungal resistance pattern of *Candida* species in the Indian population. Medical mycology. 2021 Dec;59(12):1145-65.

22) Al-shemmary II. In vitro MIC of itraconazole against different isolates of

Candida albicans. Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683-3597, E-ISSN: 2521-3512). 2011;20(1):33-7.