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Isolation And Identification of Candidal Species From Oral Cavity

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Abstract:
Introduction*: The fungi are a diverse group of saprophytic and parasitic eukaryotic organisms. The most important oral yeasts belong to the genus Candida. In recent years there has been an increased interest in infections caused by the opportunistic pathogen Candida. The growing importance of Candida is in part related to the emergence of HIV infection and the more widespread use of immunosuppressive chemotherapy. Identification of infecting strains of Candida is important because isolates of Candidal species differ widely, both in their ability to cause infection and also in their susceptibility to antifungal agents.*
Aim*:The aim of the study is to identify and isolate the candidal species in dental patients by culturing them using Sabouraud's Dextrose Agar and CHROMagar medium*
Materials And Methods*: A cross sectional study was done for a period of four months in which a total of 40 subjects 10 of them presenting with clinical oral manifestations of candidiasis, 10 of them who were denture wearers, 10 of them with poor oral hygiene, 10 patients with reasonably good oral hygiene were selected for the study were included in the study after obtaining informed consent from the Outpatient Department of Oral Medicine and Radiology, Meenakshi Ammal Dental College And Hospital, Chennai, India.*
Results*: In our study we were able to isolate six different candidal species from oral caviy of patients which comprised of Candida albicans and five nonalbicans species which included Candida tropicalis, Candida krusei, Candida dubliensis, Candida parapsilosis and Candida glabrata. We found a statistically significant p value of less than 0.05 in assessing the various objectives in different species as a whole, but when analysed individually there was statistically significant differences observed in only two species C.albicans and C.tropicalis.*
Conclusion*:On the whole it was observed that as the CHROMagar medium gives a presumptive identification within 48 hours, preliminary antifungal treatment can be administered with confidence while the confirmed identification is being obtained. The availability of this type of media not only facilitates the provision of rapid patient care, but may also assist to control the rise in antifungal agent resistance by reducing the time taken for presumptive identification of the organism at species level to start the therapeutic regime.*
Keywords: *Candida, Oral Cavity, Opportunistic, Isolation, Dental Patients, Sabouraud's Dextrose Agar and CHROMagar Medium*

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I. Introduction

Colonization of the mouth by Candida species has a long recorded history. Hippocrates, as early as 377 BC, reported oral lesions that were probably caused by Candida (Odds- 1988).[12] Candidiasis is caused by yeast like fungus, Candida (Monilia) albicans. These fungi are a diverse group of saprophytic and parasitic eukaryotic organisms. In recent years there has been an increased interest in infections caused by the opportunistic pathogen Candida. The growing importance of Candida is in part related to the emergence of HIV infection and the more widespread use of immunosuppressive drugs. Identification of infecting strains of Candida is important because isolates of Candidal species differ widely, both in their ability to cause infection and also in their susceptibility to antifungal agents. Although other species, such as *C.tropicalis, C.parapsilosis, C.stellatoidea, C.krusei, C.gulliermondii, C.dubliniensis* and *C.glabrata* may also be involved. Candida exists in three forms namely pseudohyphae , yeast and chlamydospore forms. It reproduces by asexual budding and forms pseudohyphae .These species grow rapidly at 25-37^0 C. It has been shown that this microorganism is a relatively common inhabitant of the oral cavity, gastrointestinal tract and vagina of clinically normal individuals. Thus it appears that, mere presence of the fungus is not sufficient to produce the disease. There must be actual penetration of the tissues, although such invasion is usually superficial and occurs only under certain

circumstances. This disease is said to be the most opportunistic infection in the world.[2]Wearing removable dental prosthesis causes an alteration in the microflora. For certain individuals, this new environment is responsible for the development of a particular condition: denture stomatitis, or denture associated stomatitis. The *Candida* species, in particular is known to increase with the use of dentures. The combination of entrapment of yeast cells in irregularities in denture relining materials, poor oral hygiene and several systemic factors is the most probable cause for the onset of the disease The most common procedures are microscopic examination and culture. A few laboratories still prefer to use conventional methods to identify yeasts, such as the use of, Sabouraud's Dextrose Agar, germ tube test, cornmeal agar, carbohydrate utilization and carbohydrates fermentation tests. In order to facilitate rapid identification, alternative techniques to standardize, media such as chromogenic media have been developed. These special media yield microbial colonies with varying pigmentation secondary to chromogenic substrates that react with enzymes secreted by the microorganisms. CHROMagar *Candida* (CaC) employs this methodology to allow the differentiation of several candidal yeasts by colour and morphology; it identifies *C.albicans* by growth as green colonies ,*C.tropicalis* by growth as steel blue colonies and *C.krusei* as rough, matted rose coloured colonies. Other species such as *C.glabrata* and *C.dubliensis* may be identified by white and brown colours reliably (Hospenthal *et al* - 2012, Kirkpatrick *et al* – 1998, Odds & Bernearts – 1994, Odds & Davidson -2000, Pfaller *et al* -1996).[10-13]With this background, a study is planned to isolate, identify the various species of *Candida* from the oral cavity by using Sabouraud's Dextrose Agar and CHROMagar medium.

II. Materials And Methods

A prospective, comparative study was done for a period of four months in which a total of 40 subjects 10 of them presenting with clinical oral manifestations of candidiasis, 10 of them who were denture wearers, 10 of them with poor oral hygiene, 10 patients with reasonably good oral hygiene were selected for the study after obtaining informed consent from the Outpatient Department of Oral Medicine and Radiology, Meenakshi Ammal Dental College and Hospital, Chennai, India.

2.1 Inclusion Criteria

1. Patients in the age group of 20 to 80 years.
2. Patients wearing partial or complete dentures.
3. Patients presenting with candidiasis including acute pseudomembranous candidiasis, erythematous (acute/chronic), chronic hyperplastic candidiasis.
4. Patients with candida-associated lesions like denture stomatitis, angular cheilitis, median rhomboid glossitis.
5. Patients undergoing long term steroid and antibiotic therapy.
6. Patients undergoing radiotherapy.
7. Patients with poor oral hygiene.
8. Patients with reasonably good oral hygiene
9. Patients with known history of controlled diabetes mellitus and hypertension .
10. Patients who were willing for the study

2.2 Exclusion criteria

1. Patients who were under antifungal drugs.
2. Patients who had restricted mouth opening.
3. Patients who were not willing for the study

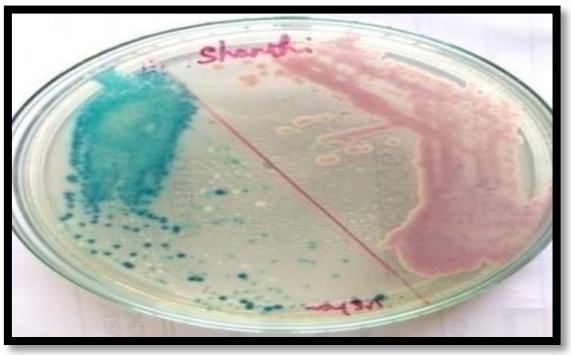
III. Swab Collection Method

The sampling approach involves gently rubbing a sterile cotton swab over the lesion and then subsequently inoculating a primary isolation medium such as Sabouraud's dextrose agar (SDA)followed by culturing it in CHROMagar medium.

Sterile swab collection tube

Sample Collection done using sterile swab from the denture bearing area of the palate

The sampling approach involves gently rubbing a sterile cotton swab over the lesional tissue and then subsequently inoculating a primary isolation medium such as Sabouraud's dextrose agar (SDA). Typically SDA is incubated aerobically at 37◦C for 24–48hrs. *Candida* develops as cream, smooth, pasty convex colonies on SDA which is later transferred to the CHROMagar medium.

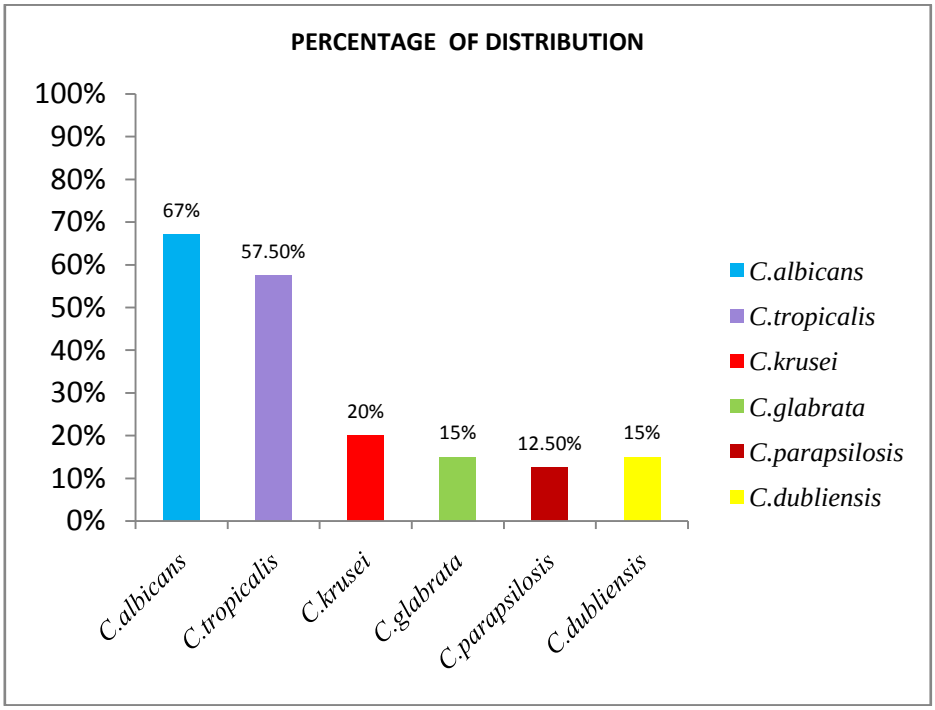


Growth of Candidal Colonies on HiCHROMagar medium

IV. Results

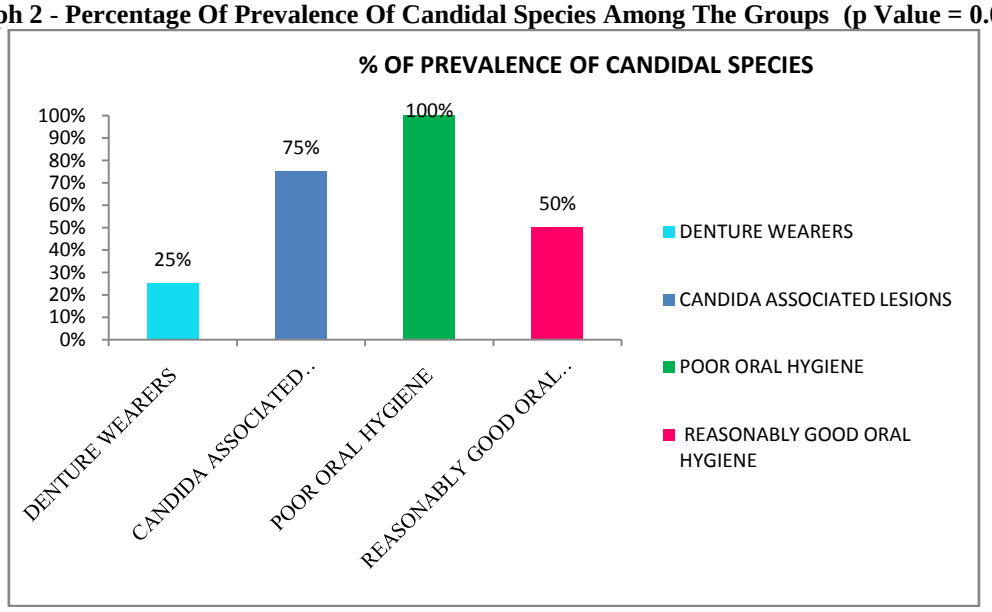
The present cross-sectional study aimed at the isolation and identification and speciation of *Candida* from oral cavity using the swab technique in denture wearers, *Candida* associated lesions, reasonably good oral hygiene and poor oral hygiene patients using CHROMagar medium .The mean age of the patients included for the study was 49.72 years. In our study we were able to isolate six different candidal species namely *C.albicans, C.tropicalis, C.krusei, C.glabrata, C.parapsilosis* and *C, dubliensis*. The results of this study were subjected to statistical analysis by employing Chi square test, to evaluate the prevalence of various candidal species in different groups, influence of systemic diseases /conditions on the prevalence of candidal species, influence of deleterious habits on candidal species and comparative analysis of prevalence of candidal species among gender using SPSS (statistical package for social science) software version 26.We found a statistically significant p value of less than 0.05 in assessing the various objectives in different species.

Graph 1-Percentage Of Prevalence Of Candidal Species



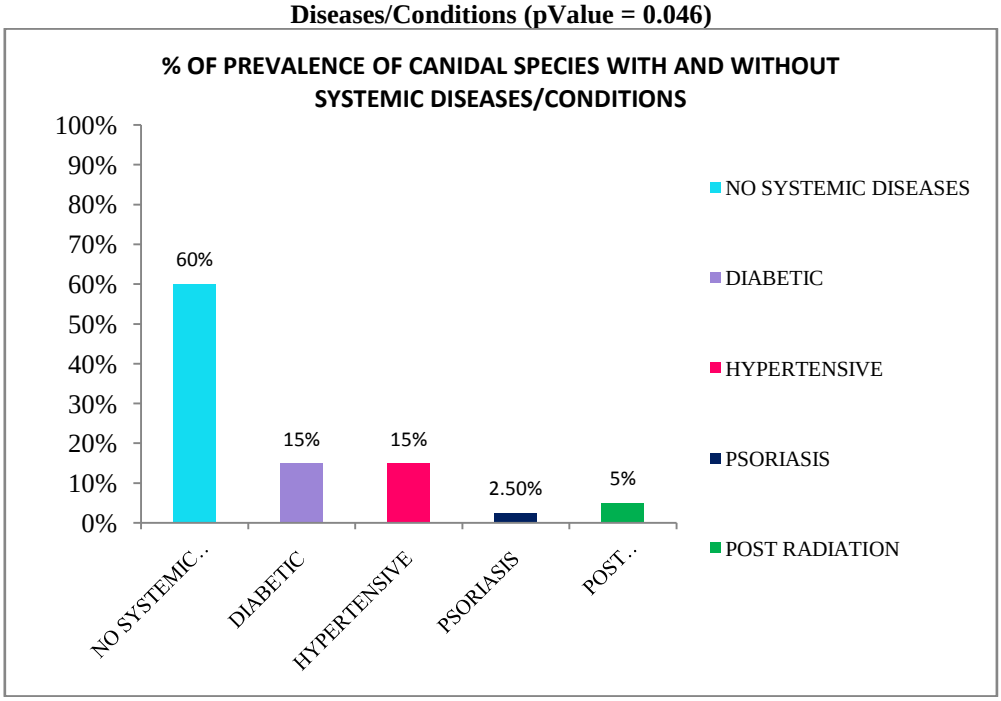
The percentage of prevalence of candidal species were analysed and we found that *C.albicans* was present in 67% followed by *C.tropicalis* in 57.5%, *C.krusei* in 20%,C.glabrata and *C.dubliensis* showing equal prevalence of about 15% and *C.parapsilois* in 12.5% of the patients (Graph-1).

Graph 2 - Percentage Of Prevalence Of Candidal Species Among The Groups (p Value = 0.032)



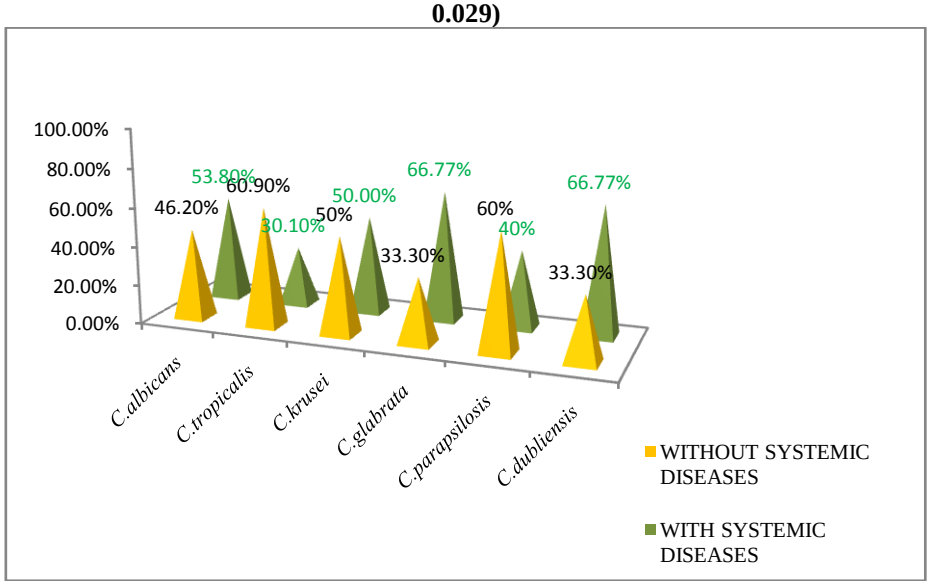
In our study we were able to observe that prevalence of Candidal species in poor oral hygiene group was found to be 100% followed by 75% in Candida associated lesion group, 50% in reasonably good oral hygiene group and only 25% in denture wearers (Graph 2).

Graph 3- Percentage Of Prevalence Of Candidal Species With And Without Systemic Diseases/Conditions (pValue = 0.046)



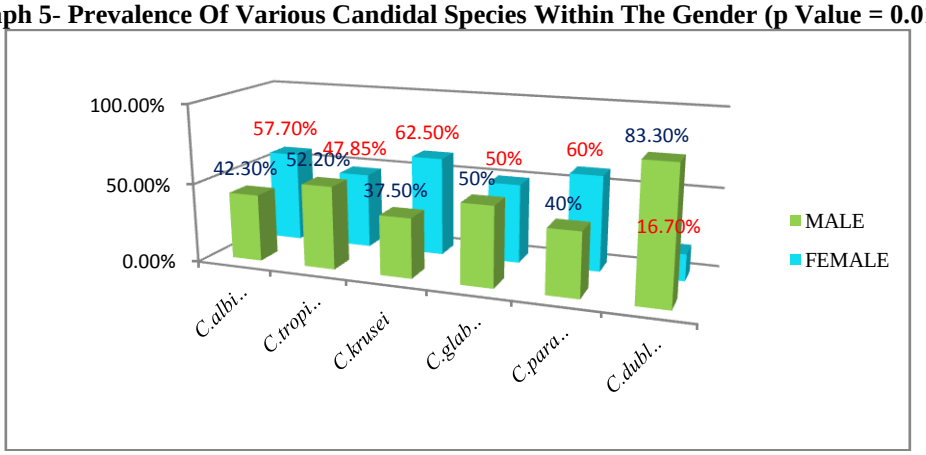
Influence of systemic diseases and conditions on the candidal growth was analysed in our study .We found that 60% of the patients had no systemic disease or conditions and 40% of them had some kind of systemic disease or condition. Out of 40% of the patients who exhibited candidal growth, 15% were diabetic and 15% were hypertensive and 5% of patients who had post radiation xerostomia exhibited candidal growth (Graph 3). We assessed the influence of systemic disease on the prevalence of different candidal species of which *C.albicans, C.glabrata* and *C.dubliensis* was found more in patients without systemic diseases whereas *C.tropicalis, C.parasilosis* and *C.dubliensis* was found more in patients with systemic diseases.

Graph 4- Prevalence Of Candidal Species In Patients With And Without Systemic Diseases (pValue = 0.029)



We assessed the influence of systemic disease on the prevalence of different candidal species of which *C.albicans* (53.80%), *C.glabrata*(66.77%) and *C.dubliensis(66.77%)* was found more in patients with systemic diseases whereas *C.tropicalis* (60.90%) *and C.parasilosis* (60%) (Graph 4).

Graph 5- Prevalence Of Various Candidal Species Within The Gender (p Value = 0.017)



In our study the prevalence of various species among the gender was evaluated. We found that *C.albicans* was found more in female patients which was (57.70%) and 42.30% in male patients. *C.tropicalis* and *C.glabrata* was found in equal predliction among the males and female subjects. *C krusei* was found more in female patients which was 62.5% and 37.5% in male patients. *C.dubliensis* was found more in male patients (83.3%) than female patients (16.7%)(Graph 5).

V. Discussion

In this cross sectional study done over a period of four months time in patients attending the Dental Outpatient Department, we aimed at isolation and identification of oral candidal species in four different groups of patients. The different groups of patients included in the study were the denture wearers, patients with candidiasis and its associated lesions and the third group comprised of patients with poor oral hygiene fourth group of patients had reasonably good oral hygiene.A study done by Sayyadha *et al* in (2010)[42] and Murray *et al* in (2005)[43] three species of *Candida* in CHROMagar medium were identified on the basis of colony colour and morphology, and accurately differentiate between them i.e. *Candida albicans*, *Candida tropicalis*, and *Candida krusei* . In previous studies done by Penha SS (2000) [23] ,Zaremba ML *et al* (2006)[24] and Mizugai *et al* (2007)[18] they were able to obtain only four different species namely *C.albicans*, *C.tropicalis*, *C.krusei* and C.glabrata. whereas in our study we were able to isolate six different candidal species namely *C.albicans*, *C.tropicalis*, *C.krusei*, *C.glabrata*, *C.parapsilosis* and *C. dubliensis.* we found that *C.albicans* was present in 67% followed by *C.tropicalis* in 57.5%, *C.krusei* in 20%,C.glabrata and *C.dubliensis* showing equal prevalence of about 15%

and *C.parapsilois* in 12.5% of the patients.Gasporoto TH et al (2009)[38] identified C.dubliensis as a additional species along with C.albicans, in both denture wearers and non denture wearers but in our study we were able to isolate *C.dubliensis* in all four groups.

Isolation and identification of candidal species was carried out by utilizing the conventional technique i.e. Sabouraud's dextrose agar (SDA) and the CHROMagarcandida. Growth was observed in 34 out of 40 samples using primary medium (SDA. In our studyin the denture wearers group complete denture wearers were 40% and partial denture wearers were 60%. In this study 70% of the denture wearers exhibited candidal growth 30% of the patients showed no growth. This finding coincided with Arendrof and Walker (1980) where the colonization in clinically normal mouths of healthy adults ranged from 3 to 48%.[19] A variation in candidal growth was observed in healthy population by various authors in their studies. In our study candidal growth as observed in 90% of the patients who were denture wearers which coincided with the study done by Lockhart SR *et al* (1999)[37] in denture patients showed a growth in almost ranging from 50 to 92% and study done by Daniluk *et al* (2006)[3] and Gasparoto HT *et al* (2009)[38] showed growth in denture wearers with stomatitis to be 75% which was slightly lower when compared with our study.

The study conducted by Ikebe K *et al* (2006)[28] showed a high candidal activity in male denture wearers. In our study all patients with maxillary dentures exhibited candidal growth which coincided with the study conducted by Budtz- Jorgensen E (1981)[21], the increased colonization of palatal mucosa could be due to slight elevation of temperature beneath the denture producing a thermal irritation, decreased pH due to increased consumption of carbohydrate and constant wearing of the denture which may stimulate multiplication of microorganism. In our study, the candidal growth was increased in the non denture wearers than denture wearers, similar to the findings of Narhi TO *et al* (1993)[39], Ikebe K *et al* (2006)[25], Gasparoto HT *et al* (2009).[38] In patients with poor oral hygiene Almost all the patients exhibited positive candidal growth which was in accordance with the study conducted by of Narhi TO *et al* (1993)[67] and Ikebe K *et al* (2006)[25].

A number of sampling methods for the identification and isolation of candidal organisms have been utilized. In majority of the clinical investigations, yeasts were routinely cultured on Sabouraud's dextrose agar. General purpose of this medium is to support the growth of pathogenic fungi. Sabouraud's dextrose agar is not a differential medium hence it is difficult to distinguish different species of *candida* using this medium. So further differentiation of species has to be carried with CHROMagar medium. In this CHROMagar medium the differentiation of various species are carried out based on the contrasting colony colours produced by species specific enzymes with a proprietary chromogenic substrate .The medium greatly facilitates the detection of specimens containing mixture of yeast species. The presence of Hexoseaminidase enzyme activity of the candidal organism and the production of specific colour by different candidal subtypes was the basis of using CHROMagar (Odds FC and Bernets R 1994)[12] As per a study done by Duane R. Hospenthal *et al* (2002) mixtures of three isolates of *candida* were harder to distinguish which is in contradiction with our study as we able to identify different candidal species clearly.

VI. Conclusion

On the whole it was observed that as the CHROMagar media gives a presumptive identification within 48 hours, preliminary antifungal treatment can be administered with confidence while the confirmed identification is being obtained. The availability of this type of media not only facilitates the provision of rapid patient care, but may also assist to control the rise in antifungal agent resistance by reducing the time taken for presumptive identification of the organism at species level to start the therapeutic regime. We can conclude that use of fast and accurate diagnostic methods can help in rapid treatment of patients.

VII. Future Prospect of This Study

The study has to be carried forward to assess the antifungal susceptibility testing of the various candidal species to different antifungal drugs in order to obtain an idea regarding which species would respond to which family of antifungal drug and to establish a guideline to treat patients with antifungal drug appropriately.

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