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Surveillance of upper aerodigestive candidiasis and their antifungal susceptibility study at tertiary care hospital: A prospective study

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ABSTRACT

Candida is one of the most common problems faced these days which require timely medical attention. We have aimed to identify the *Candida* albicans and non-albicans species of upper aerodigestive fungal infections and their antifungal drug's susceptibility using various antifungal. Swab samples were collected from the upper aerodigestive area and by use of Sabouraud dextrose agar to identify Candida fungal growth. Albicans and non-albicans Candida species were identified by the use of candida differential agar media. Disk-diffusion method is used to evaluate antifungal susceptibility. Two hundred and fifty-two samples were identified with candidiasis. This study found that *Candida* albicans species are more in comparison to non-albicans species with a female predilection in upper aerodigestive candidiasis. All *Candida* isolates species showed the least resistant to itraconazole and amphotericin B whereas 60% maximum resistant to fluconazole by *Candida tropicalis* than 54% resistance to clotrimazole shown by *Candida glabrata*, resistance to nystatin 50% by *Candida krusei,* and resistance to ketoconazole 24% by *C. tropicalis*. *Candida parapsilosis* showed 100% susceptibility to miconazole, itraconazole, and amphotericin B. So, suggesting that empirical use of antifungal can be harmful to the patient and can lead to the growth of further development of more resistant strains of Candida and this study revealed that in Itraconazole and Amphotericin B is the most effective antifungals for upper aerodigestive candidiasis in comparison to fluconazole.

1. INTRODUCTION

Fungal infections, especially candidiasis caused by *Candida,* are one of the most common problems faced these days which require timely medical attention. The frequency of these infections increases furthermore due to the currently extended lifespan of the common population which includes the elderly and those who are immune compromised. Fungal infections may lead to fatal conditions such as fungemia and meningitis. Millions of people suffer from fungal infections annually [1-5].

Candida is the causative agent of the most common invasive fungal infection occurring in developed as well as developing countries. Diagnosis of candidiasis is usually delayed due to lengthy procedures like blood cultures which have low sensitivity. This is also because fungal-origin infections are suspected when the fever does not subside even after taking antibiotics [5,6]. There are around 30 species of *Candida* causing candidemia, but the most common among them are *Candida* albicans, *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis* constituting 80% of the total *Candidal* infections [7,8]. *Candida* albicans is the most common opportunistic pathogen which is normally present in the oral cavity, conjunctiva, gastrointestinal, and genitourinary tracts. Invasive candidiasis caused due to *Candida* albicans amounts to 50–70% of total clinical cases. The four important antifungal drugs used at present times are azoles, polyenes, echinocandins, and pyrimidine analogs [9-11]. However, fatal prognosis if the pathogen is found to be resistant to one or more of the above antifungal agents. The problem gets further intensified as different Candidal species show diverse resistance patterns to various antifungal drugs. Hence, it is advisable to identify fungal strain, as well as have to perform the susceptibility test, to decide the appropriate choice of treatment [12-15]. Furthermore, one needs to consider the acquired resistance of the various species of *Candida* which is

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evolutionary leading to a lowering of susceptibility to a particular drug while treating an infection [13]. The above may be acquired by mutations and chromosomal rearrangements. Increasing resistant strains which include strains that are resistant to multiple drugs are now being reported all over the world through various studies [16-20].

The resistant phenotypes emerge during the period of the disease and also as a result of a particular treatment which can complicate the problem further [6,7]. As various factors (patients' immune condition, virulence factors of candida) related to emergence of drug resistance of different types ofantifungal drugs, so, this study should be done from time to time to evaluate candida species involvement and drug susceptibility. Hence, our study aimed to evaluate antifungal resistance to different antifungals. It has also been seen that patients suffering from systemic diseases like diabetes show increased colonization of *Candida*. Other conditions such as neutropenia, malignancies treated with chemo/radiotherapy, or immunocompromised state arising due to long-term dose of steroid or antibiotics also show increased colonization of *Candida* [21-25].

This study was approved by the Institutional Ethical Committee (Ref. No. DMR/IMS.SH/SOA/180254), IMS and SUM Hospital, Siksha "O" Anusandhan University, Bhubaneswar. This study aimed to identify the antifungal susceptibility of *Candida* species isolates from the upper aerodigestive tract (oral and oropharyngeal) *Candida* infections.

2. MATERIALS AND METHODS

In this prospective study, all the patients attending to hospital for 2 years from January 2018 to Dec 2019 were included in the study. OPD patient who has local (poor oral hygiene, on inhaled steroid therapy, ill-fitting denture, and high sugar diet) or systemic predisposing factors (malnutrition, old age, iron deficiency, an endocrine disorder, on long-term antibiotics, or steroid therapy) for oral and oropharyngeal candidiasis were included in this study, whereas HIV patients were excluded in this study to confine the study to OPD group of patients. Three hundred samples were collected from the otorhinolaryngology department and dental department in a tertiary care teaching hospitals of Eastern India, Odisha.

Swab samples were collected from oral and oropharyngeal lesional area [Figure 1]. Collected samples were processed for fungal culture by inoculation in SDA (Sabouraud's Dextrose Agar) medium. In an aerobic environment, plates were incubated at 37°C for 24 h after overnight incubation and colonies of *Candida* colonies were obtained. By use of *Candida* differential agar media, different colonies were identified on the basis of color (Light green color of Candida albicans, blue to purple color of *C. tropicalis*, cream to pinkish-white color of *C. glabrata*, fuzzy purple color of Candida krusei, and white to cream color of *C. parapsilosis*). With the use of various antifungal disk (Fluconazole (FLC^{10}), nystatin (NS^{50}), amphotericin B (AP^{20}), itraconazole (IT^{30}), ketoconazole (KT^{50}), miconazole (MIC^{50}), and clotrimazole (CC^{10}) by disk-diffusion method, antifungal susceptibility test was carried out and inhibition zone was measured by following criteria of Hi-media, to know antifungal drug's resistance and susceptibility [Figure 2].

3. RESULTS

We have evaluated the *Candida* species and the drug's susceptibility to identified *Candida* species, of upper aerodigestive (oral and oropharyngeal) fungal infections.

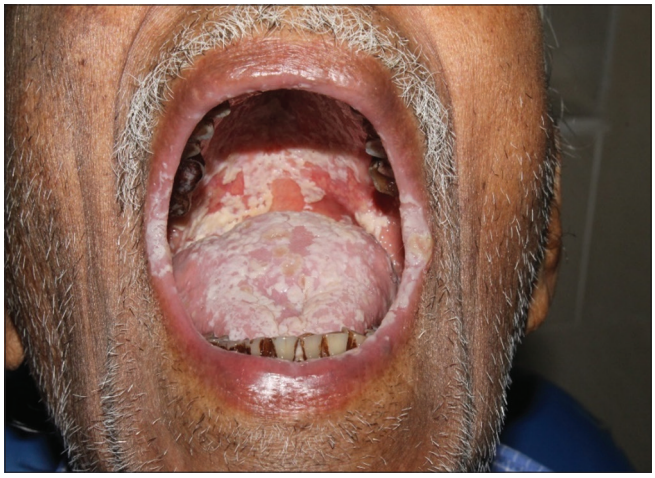

Figure 1: Oral and oropharyngeal candidiasis.

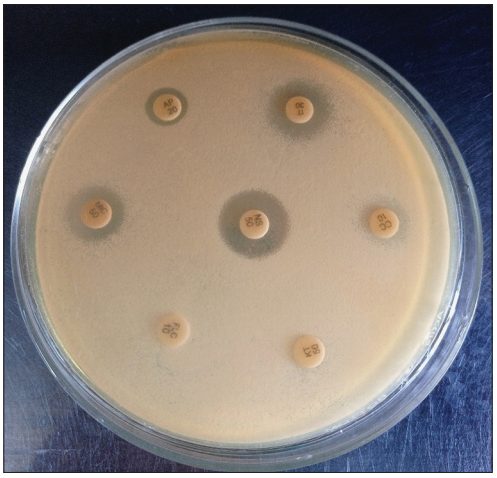

Figure 2: Antifungal susceptibility test by disk-diffusion method.

Out of 300 samples, 252 samples were positive for *Candida* infections. Further species identification on CDA agar done and found 137 samples as *Candida* albicans whereas 115 samples were non albicans candida species. The most prevalent species about 54.3% cases were *Candida* albicans, whereas 19% were *C. tropicalis*, 16.6% were *C. glabrata*, 6% were *Candida krusei*, and 4% cases were *C. parapsilosis.* The most abundant species among non-*Candida albicans* species was *C. tropicalis.* It has been also observed that *Candida* infections were seen more during September to December month may be due to the cold winter season one of the reasons for the occurrence of more infectious diseases and more use of higher antibiotic and weak immunity of individual can lead to candidiasis [Table 1]. Females' patients showed more fungal infection with *Candida* albicans, *C. tropicalis*, *C. glabrata,* and *Candida krusei* as compared to the male patient [Table 2]. Regarding age group involvement ≥60 years age group of patients showed comparatively higher upper aerodigestive candidiasis infection (38.5%) in comparison to the adult age group (31.3%) [Table 3]. Figure 2 shows the disc diffusion method of the antifungal susceptibility test of a sample. Antifungal susceptibility test suggests that all *Candida* isolates species show the least resistance to itraconazole and amphotericin B whereas more resistant 60% to fluconazole by *C. tropicalis* than resistance to clotrimazole 54% by *C. glabrata*, resistance to nystatin 50% by *Candida* krusei, and resistance to ketoconazole 24% by *C. tropicalis*. *C. parapsilosis*

Table 1: Timeline for *Candida* species (candida albicans and non-albicans) isolated from upper aerodigestive tract.

Period of Sample collection	*Candida* albicans	*Candida tropicalis*	*Candida glabrata*	*Candida*. krusei	*Candida parapsilosis*
January–March 18	10	10	4	1	0
April–June 18	12	8	03	0	0
July–September 18	20	2	1	1	0
October–December 18	22	4	12	2	0
January–March 19	20	8	10	4	5
April–June 19	12	10	6	7	3
July–September 19	17	5	1	0	2
October–November 19	16	1	2	0	0
December 19	18	0	3	0	0
Total=252	137 (54.36%)	48 (19%)	42 (16.6%)	15 (5.9=6%)	10 (3.9=4%)

Table 2: Gender-wise distribution of *Candida* albicans and non-albicans species.

Candida species	Male	Female	Total	% of candida species species
Candida albicans	63	74	137	54.36
Candida tropicalis	21	27	48	19
Candida glabrata	19	23	42	16.6
Candida. krusei	7	8	15	6
Candida parapsilosis	6	4	10	4

Table 3: Occurrence of candida in different age group.

Age groups	Male	Female	Total 252
Children (0–18)	35	41	76 (30.1%)
Adult (19–59)	30	49	79 (31.3%)
Senior (old age ≥60)	45	52	97 (38.5%)

showed 100% susceptibility to miconazole, itraconazole, and amphotericin B [Table 4].

4. DISCUSSION

Candida species are found in the human body normally as human flora at various sites such as in the skin, oral cavity, gastrointestinal tract, and genital tract. However, under special circumstances like elderly patients, hospitalized and immunocompromised patients show growth of this opportunistic candida infection so there is the chance of invasive candidiasis in this group of patients. Proper laboratory setup is must otherwise led to false-positive results and most of the time, empiric antifungal therapy has become the choice of treatment in most patients having fever and septic infections or patients who have undergone surgeries with a venous catheter or under chemotherapy. One of the causes of rise in the resistant strains of fungi is due to undue usage of antifungal that further leads to delay in the treatment procedure for the patients. The treatment outcome of candidiasis is decided by various factors such as the immunity of the patient, severity of infection, and site of infection. The four important antifungal agents which are used these days are polyenes, azoles, echinocandins, and 5-Flucytosine. However, if antifungal resistance developed during treatment still poses a serious threat to the health of human populations [26-33].

In this study, the susceptibility *in vitro* of *Candida* strains was tested for amphotericin B, ketoconazole, clotrimazole, fluconazole, itraconazole, miconazole, and nystatin. The incidence of fungal infections has drastically increased over the past three decades and was simultaneously accompanied by increased acquired and innate resistance to antifungal drugs. However, epidemiological data regarding the incidence of resistance among fungal species are not identically distributed worldwide. According to most of the surveys done in the world, different susceptibility patterns of *Candida* species have been observed. *C. glabrata* and *Candida* krusei have been found to show low susceptibility to azoles whereas *C. parapsilosis* has low susceptibility to echinocandins. Minimum inhibitory concentration is designated as the lowest concentration of the tested compound, which is used to assess the level of susceptibility. Most laboratories nowadays do the disk-diffusion method in routine practice to analyze antifungal resistant of various candida species. The pattern of resistance shown by *Candida* in the globe is not a constant one as there is a rise in the resistant isolates constantly. It has been shown that the resistant species and increases after long-term use of a particular antifungal drug [31-36].

It has also been seen that the species resistant to a particular drug simultaneously develops resistance to other drugs leading to multidrug resistance (MDR). For example, *C. glabrata* and *Candida krusei* have low susceptibility toward azoles and develop resistance to echinocandins. Furthermore, the intensive use of antifungal in the past 15 years has led to an increase in MDR, especially in *C. glabrata* [7,22]. Hence, we have attempted to conduct a study to find out the susceptibility and resistance of the various *Candida* and non-*Candida* species to the various antifungal drugs commonly used in the Odisha population to see their results in the said population.

The most prevalent species was *Candida* albicans followed by *C. tropicalis* and the resistance of *C. tropicalis* isolated was highest for fluconazole and the lowest for itraconazole and amphotericin B which are in concordance with Clinical Practice Guideline for the Management of Candidiasis where suggested that fluconazole is less recommended [13,33]. As per the clinical experience of Santosh Kumar Swain [14] (ENT Specialist), itraconazole is more effective for treating upper aerodigestive fungal infections [34]. Amphotericin B is also effective, but its use should not be exceeded as it causes a nephrotoxicity effect. Although the first line of treatment of oropharyngeal candidiasis as topical (nystatin, clotrimazole, and amphotericin B oral suspension) and systemic oral azole (itraconazole, fluconazole, and posaconazole) [35,36] as increasing resistant of candida species toward fluconazole, so itraconazole should be advised. Herein, we reported resistance percentage to the various drugs such as itraconazole, amphotericin B, fluconazole, clotrimazole, miconazole, nystatin, and ketoconazole. The various *Candida* species vary in

Table 4: Antifungal screening Profile of candida albicans and non-albicans species for all tested antifungal drugs.

Antifungals *Candida* Sp.	Itraconazole IT[30]	Ketoconazole KT[50]	Amphotericin B AP[20]	Nystatin NS[50]	Fluconazole FLC[10]	Clotrimazole CC[10]	Miconazole MIC[50]
C. albicans n=137	6.25%	12%	6.25%	45%	52%	42%	12%
C. tropicalis n=48	6.25%	24%	6.25%	45%	60%	42%	12%
C. glabrata n=42	6.25%	6.25%	6.25%	45%	52%	54%	6.25%
C. krusei n=15	6.25%	12%	6.25%	50%	38%	42%	6.25%
C. parapsilosis n=10	0	12%	0	12%	34%	32%	0

their susceptibility to the available antifungal agents. The intrinsic resistance to antifungal therapy observed in some species, along with the development of acquired resistance during treatment in others, is becoming a major problem in the management of *Candida* infections. Antifungal susceptibility testing has therefore become essential for effective patient management and resistance surveillance. Many studies carried out in the past have shown different drug resistance patterns for *Candida* [26,31-36].

Hence, we emphasize that a better understanding of the mechanisms and clinical impact of antifungal drug resistance is essential for the efficient treatment of patients with *Candida* infection and for improving treatment outcomes.

5. CONCLUSION

With this study, it is revealed that oral, oropharyngeal fungal infection shows variable resistance and susceptibility to antifungal. So before prescribing the antifungal, the clinician should advise for a culture susceptibility test so that the effective drug and right drug can be prescribed.

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7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

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11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

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