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# A study on fungal speciation and drug susceptibility testing

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ABSTRACT

Fungal infections affect many people but most of these do not come to light as they are mild in clinical presentation. Candidiasis is the frequent fungal infection involving mucosa, skin, nails and internal organs caused by different species of Candida and Candida albicans being the prototype. The clinical manifestations vary with duration and severity. It occurs mostly as a comorbid disease with a primary disease or disorder. Candida comes under the phylum Fungi Imperfecti, order Moniliales and family Cryptococcaceae. Genus Candida comprises of 20 important species recognised as pathogenic in humans, of which 7 are renowned opportunistic pathogens. The following are some of the known species: Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata, Candida guilliermondii, Candida parapsilosis, Candida lusitaniae, Candida kefyr, Candida rugosa, Candida dubliniensis and Candida viswanathii. Among all the fungi, 600 species are identified to be causing infections in human. Candida albicans being one of the normal human commensals may cause infections from mild to severe forms, which is influenced by molecules that helps in adhesion and invasion, hydrolases, yeast to hyphal transition, biofilms etc. In general, equal importance has not been give to fungal infections as is being given for bacterial infections. Nowadays newly emerging species are on the tract as to cause infections and their identification profile being indeterminate, which could be confirmed only by molecular methods. Overall C.tropicalis was the frequent species causing infections clinically and resistance was demonstrated against azoles and caspofungin by C.albicans and C.krusei. This may be due to extensive use of echinocandins as empirical therapy without susceptibility testing which can increase the development of resistance and may deduct treatment options. Hence, routine antifungal susceptibility testing has to be done. Out of all these things accurate epidemiological analysis and data can be provided regarding the burden of fungal infections around the globe.

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## INTRODUCTION

In this section presents introduction of this research work. Fungal infections affect many people each year, but most of these do not come to light as they are mild in clinical presentation. Though most of them are mild, millions of people are acquiring infections that are severe and may even lead to death similar to tuberculosis. [1, 2] The invasiveness of fungus have been increasing because of the host's compromised immune status due to underlying disease or newer therapeutic changes. Since most tests

are not highly specific or sensitive and diseases are subclinical, it delays or makes difficult to diagnose and treat in time which may not bring down the morbidity and mortality rate. [3, 4] C.albicans usually colonises the oral cavity of three-fourth of normal humans, whereas other Candida non-albicans are less common. It causes infections when the immune system is compromised, until then it maintains a symbiotic relationship. [5, 6] The infection of oral cavity manifests as oral candidiasis which can further affect oropharynx and esophagus. The predisposing causes can be HIV infection, diabetes mellitus, old age etc. Candida sometimes causes dreadful mucocutaneous and cutaneous diseases. It also resides in the gastrointestinal and reproductive tract. [7, 8] The pathological course of the infection is based on host's susceptibility and type of presentation. Genitourinary infection is the common one which is of two forms, vulvovaginal candidiasis and candiduria. The fungal infections are significantly noted due to increase in various immune compromising diseases like acquired immune deficiency syndrome(AIDS) and newer therapeutic interventions like cancer therapy, transplantation etc. There are a variety of unique interactions between the parasites and the host's mucosal surfaces which exists as mutualistic relation by commensal and as parasitic relation by pathogens. [9, 10] This plays a major role in influencing progression and prognosis of the disease.

In this paper presents section 2 of this paper explains the detail on the related works. In section 3 presents the materials and methods adopted and section 4 presents the details of the experiments and discussions. Finally section 5 concludes the paper by sharing our inferences and future plans.

## RELATED WORKS

In this section presents focuses the related works of this research work. Out of two hundred species only twenty are identified to be in association with diseases in humans, of which some are major pathogens in common. Those species are

- Candida albicans,
- Candida glabrata,
- Candida dubliniensis,
- Candida guilliermondii with its teleomorphic state- Pichia guilliermondii,
- Candida krusei with its teleomorphic state: Issatchenkia orientalis,
- Candida lusitaniae with its teleomorphic state-Clavispora lusitaniae,
- Candida kefyr with its teleomorphic state: Kluyveromyces marxianus,
- Candida tropicalis and
- Candida parapsilosis.

This infection was observed to be most frequent manifestation among the cutaneous candidiasis [11, 12] . Intertrigo is characterised by dermatitis with superficial inflammation that occurs in skin surfaces which are close in opposition resulting in friction, moisture and poor ventilation as predisposing factors. The inflammation in skin is made worse or aggravated by feces, urine, sweat and other body fluids/secretions. On examination physically the skin folds shows erythema scaling peripherally. Due to exceeding friction with inflammation it can lead to skin damage and can give way for secondary infections even by bacteria. Intertrigo shows unique pattern of satellite lesions with which the infection is diagnosed. Intertrigo can occur in acute cutaneous infections displaying intense erythema, creamy exudate, edema and satellite-like pustules in the skin folds [13, 14]. Interdigital candidiasis: This infection can be chronic with infected stratum corneum as thick white layer upon epidermis in the interdigital spaces [15]. In interdigital candidiasis the skin folds of the fingers are affected with maceration and itching. This form has an association especially with fruit cannery workers, dishwashers and bartenders who are exposed to moisture excessively. This also occurs in warm seasons with same erosive rash [16].

Perianal(diaper) rash: This is otherwise known as diaper or napkin dermatitis which present as contact dermatitis with acute inflammatory irritation on the diaper region. It is a frequent dermatological disease occurring in children and infants. The causation was believed to be ammonia in the past but other factors like wetting, friction, improper skin care, antimicrobial agents, microorganisms and lack of nutrition was came to know later. The common parts involved in this dermatitis are thighs, lower part of abdomen and the diaper region. It typically involves skin folds and in the initial phase of the disease only dryness occurs where it later turns into erythema with edema and maceration in the affected region. This dermatitis can be complicated with secondary bacterial and fungal infections [17].

Candidids: This disease form is less common and the lesions that appear is based on the immune response of the host against the infection (candidids). It was taken up as an allergic response similar to dermatophytids (dermatophytic infection) which is less commonly noted. This candidids reaction disappears with general treatment for candidiasis [18].

Chronic mucocutaneous candidiasis(CMC): This disease form is an infrequent superficial infection that affects nails, skin and mucosal membranes of vagina and oropharynx. CMC is frequently in association with defective functioning of cell-mediated immunity and if not treated the progression will not have slackening. Intravenous administration of amphotericin B may clear infection or it can return back to the previous state but relapse comes only when therapy is discontinued [19].

This clinical entity of infection is represented by persistent lesions, recurrence with high rate, acquiring infection from childhood and remaining throughout for the whole life [20].

Selective susceptibility for CMC is seen in rare patients suffering from infections with persistence and recurrence along with severe debilitating property. From this knowledge it was termed as chronic mucocutaneous candidiasis [21].

Lesions can also appear on other skin

surfaces and not restricted to skin folds as seen in other disease forms of cutaneous candidiasis. They appear as hyperkeratotic lesions throught the body which is pronounced as 'Candida granuloma'(6). CMC is always preceded with T cell-mediated immunodeficiencies [22].

A surprising event in CMC which dragged everyone to conclude about the immune status in candidiasis was the fact of process which says the development of systemic and deep-seated infections will not occur in individuals with extensive cutaneous infections of mucosa and nail. In addition to this, patients with AIDS are also included in the same group who won't present systemic infections. This may be due to the severity and repeated occurrence of mucocutaneous infections. This observance brought an assumption of variation in immune mechanisms between mucocutaneous and deep-seated forms of candidiasis [23–25].

## MATERIALS AND METHODS

In this section presents the materials and methods of this research work. The study was conducted in Guntur during the period of 1 year from June 2018 to June 2019. This was a prospective study. Around 200 samples, which were clinically suspected to be candidiasis were included in the study. The Institutional Ethical Committee approval was obtained prior to commencement of the study. The samples included were blood, urine and exudates like wound swab, pus, high vaginal swab (inclusion criteria) and samples excluded were sputum, nail clippings, stool, CSF, pleuralfluid, peritonealfluid (exclusion criteria) (Table 1 ).

**Table 1: : Colour production in Chrom agar**

| S.no | Organism | Colour |
|---|---|---|
| 1. | Candida albicans | Light-green |
| 2. | Candida dubliniensis | Dark-green |
| 3. | Candida glabrata | Pink to purple |
| 4. | Candida krusei | Pink |
| 5. | Candida parapsilosis | Cream to pale pink |
| 6. | Candida tropicalis | Blue with pink halo |

The samples were collected after obtaining informed consent from the patients. Before isolation all the samples were subjected to Gram stain to look for fungal elements. After isolation in Sabouraud's dextrose agar, all the isolates were subjected to standard conventional methods for species identification (Table 2 ).

**Table 2: Colour production in Tritetrazolium reduction medium**

| S.no | Organism | Colour |
|---|---|---|
| 1. | Candida albicans | Cream to Pale pink |
| 2. | Candida tropicalis | Dark-maroon red |
| 3. | Candida parapsilosis | Rose pink |
| 4. | Candida krusei | White to pale-pink and dry |
| 5. | Candida glabrata | Pale pink |
| 6. | Candida guilliermondii | Pink and pasty |
| 7. | Candida pseudotropicalis(C.kefyr) | Salmon pink |

200 samples of patients suspected to be suffering from candidiasis were collected and inoculated onto appropriate media. After isolation of Candida, the isolates were sub cultured on chromogenic media, and sugar fermentation and sugar assimilation tests for speciation were performed. Then the antifungal susceptibility of each species was identified by disk diffusion method and VITEK-2 system.

## RESULTS AND DISCUSSIONS

In this section focuses the results and discussions of this research work. The 200 samples 65 Candida species were isolated. The predominant isolates were Candida nonalbicans (73.84%)

and Candida tropicalis (41.53%) was the prevalent species. The next predominant was Candida albicans (26.15%) followed by C.glabrata (13.84%), C.parapsilosis (10.76%), C.dubliniensis (4.61%) and C.krusei (3.07%). An isolate of C.albicans was resistant to fluconazole and voriconazole in disk diffusion while it was intermediate resistant to the same drugs in VITEK-2. Another isolate of C.krusei was having intermediate susceptibility to caspofungin (Table 3 ).

**Table 3: Susceptibility of Candida spp . in disk diffusion method**

| Species | Fluconazole | | | Voriconazole | | |
|---|---|---|---|---|---|---|
| | S | I | R | S | I | R |
| C.tropicalis | 27 | 0 | 0 | 27 | 0 | 0 |
| C.albicans | 16 | 0 | 1 | 16 | 0 | 1 |
| C.glabrata | 9 | 0 | 0 | 9 | 0 | 0 |
| C.parapsilosis | 7 | 0 | 0 | 7 | 0 | 0 |
| C.dubliniensis | 3 | 0 | 0 | 3 | 0 | 0 |
| C.krusei | 0 | 0 | 2 | 2 | 0 | 0 |

Candida species, being a commensal have now become one of the common opportunistic pathogens and is responsible for various invasive infections. The pattern of Candida albicans being the common species have been altered competitively and replaced by Candida nonalbicans in recent years.

The predominant species in our study was Candida tropicalis, whereas the same species was observed by Patel et al, who reported a prevalence of 40.9% in an Ahmedabad tertiary care hospital which is similar to our result of 41.53% prevalence of C.tropicalis. other studies by Kashid et al, also exhibited a prevalence of 46.25% and by Sowmya et al ( a study performed in Mysore) reported a prevalence of 50%.

Furlaneto et al in a 3 year study that analyzed the distribution of Candida species in a tertiary care in Brazil showed C.tropicalis was the predominant species even though C.albicans was also isolated frequently and the prevalence was 33.2% which was to some extent different from the findings of this study.

The common species in blood samples was Candida tropicalis (80%) which is similar to a observation in a study conducted by Vijayakumar et al, which was a molecular study with 74.35% prevalence in blood but the percentage of prevalence was having a slight variation from our study results.

Common species in urine was also C.tropicalis (35.71%), shown similarly by Yesudhason et al. a study that was conducted in southern India , where most of them are from urine but had a predominance of 63.93% which was having variation to some extent with our study prevalence . It was also said to cause hospital-acquired infections frequently and as a rising fungal species and so the result of our study can also be taken into account as evidence.

All the five positive blood samples for Candida were received from intensive care unit and the species isolated were 4 isolates of C.tropicalis and 1 isolate of C.glabrata species. Among these patients, one was undergoing dialysis for chronic renal failure. ICU patients are affected by many factors such as prolonged antibiotic use and indwelling catheters. The common risk factor is catheter, which is frequently used in ICU patients particularly central venous catheter. This route may provide transmission and cause blood stream infections. This has been described by Bo Hu et. al. ICU patients with candidemia was predominantly due to Candida nonalbicans particularly C.tropicalis. This was also reported in a study by Montagna et al, that Candida nonalbicans as common cause of candidemia with ICU patients but the prevalent species was C.parapsilosis.

The predisposing factor that was common among the candidiasis patients was prolonged usage of antibiotics with broad spectrum as shown similar by Sowmya et al. The second important factor was diabetes mellitus which can provide a polymicrobial profile and that can occur due to elevated sugar level. The next common factors were extreme age and catheterization. Other factor in females was pregnancy which can lead to asymptomatic candiduria. The female to male ratio was high (i.e) the candidial infection was common among females than males.

Pertaining to age group distribution the incidence was high in 41-50 age group and that was 34.42%. Most of the individuals in this age group were under long term antibiotic therapy which would probably lead to the occurrence of candidiasis. It was quite low in case of paediatric age group as when compared to adults.

In disk diffusion method only one isolate of Candida albicans showed resistance to fluconazole and voriconazole while it was intermediate resistant for both azoles in VITEK-2 analysis for the same isolate. Here disk diffusion method can be taken only as a preliminary testing, where further minimal inhibitory concentration should be determined by micro-broth dilution method for confirmation. The anti-fungal resistance of fungi is well determined with azole group of drugs which is related to different mechanisms such as alteration in tar-

get enzyme and in its level, reduced concentration of drug resulting from over-expression of efflux pump or the mechanisms all together .

One of the Candida krusei species isolated from urine was having intermediate resistance to caspofungin and this resistance acquired by the isolate against echinocandins can be due to mutation or substitution that modified glucan synthase, which has been proposed by Kahn et al .

Regarding the conventional methods, when chrom agar is compared to tritetrazolium medium(TTZ), the former is specific even though it has some pitfalls with similar colour production. But in the case TTZ, confusion arises as most species produced similar colour and some may not be specific. Chrom agar was also useful to identify and isolate from samples with mixed Candida species.

## CONCLUSION

Finally this work concludes that Out of all these things accurate epidemiological analysis and data can be provided regarding the burden of fungal infections around the globe. As tip of the iceberg, most of the fungal infections are not being reported as it is unnoticed without prominent clinical presentation and due to lack of complete documentation. C.tropicalis was the species frequently isolated in clinical specimens. C.albicans and C.krusei showed resistance against azoles and caspofungin. Hence many studies have to be conducted and routine antifungal susceptibility testing should be performed.

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### Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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