INTRODUCTION

Fungi are widespread in the environment. Some are related with animals and humans as commensals, but turn pathogenic or opportunistic after change of the host immune system [1]. Therapeutic applications of immunosuppressive drugs, the use of broad spectrum antibiotics in diverse clinical conditions and other predisposing factors are accountable for an increasing number of immunocompromised patients and consequent opportunistic infections globally. A weakened or impaired immune system provides positive conditions for pathogenic and non-pathogenic micro-organisms. In the last decades, the increase in fungal infections are multifactorial. Fungal infections are a pheno-complex of various serious complications and others. The reasons for fungal infections are multifactorial: better clinical evaluation and diagnosis, greater survival for patients with malignancies, chronic diseases, increasing number of transplants, complex surgical procedures, catheters, implants and use of wide spectrum antibiotics. Candida species have evolved a multitude of mechanisms to survive exposure to antifungal drugs and some of them include an over expression or mutations. Thus identification of Candida spp. at species level along with antifungal susceptibility testing becomes very essential. The accurate species identification of Candida is important for the treatment, as not all species respond to the same treatment because of the trouble of anti-fungal resistance. The aim of the present study was to know the prevalence of Candida spp. in lower socio-economic group, to isolate and speculate Candida spp. from urine specimens, to detect their antifungal susceptibility pattern.

MATERIALS AND METHODS

Study site

Total of 100 urine samples were collected from Erode district, Tamil Nadu belonging to different age groups of women ranging from 30 to 65 from January to February, 2012 using sterile urinals. All the specimens were transported immediately to the laboratory and cultured within 3 to 4 h of collection.
Isolation and characterization of Candida albicans

The samples were inoculated on the Sabouraud Dextrose Agar (SDA) medium and the plates were incubated at room temperature for 24 hours. After 24 h of incubation, the culture plates were examined for the appearance, size, color and morphology of the colonies were recorded. Wet mount preparation, Germ tube formation and growth on Hichrome Candida were carried out according to standard techniques.

Isolates that were Gram positive ovoid cells, Chlamydomoses production on Corn Meal agar, green color colonies on Hichrome Candida differential agar medium, the germ tube production at 45°C were considered as Candida albicans.

Antimicrobial Susceptibility Testing

Kirby-Bauer disc diffusion method is commonly employed for antibiotic sensitivity testing [10]. Antifungal susceptibility testing was performed by NCCLS M44-A disc diffusion method [11]. Briefly, antibiotic discs containing Itraconazole (10 mcg), Ketoconazole (10 mcg), Clotrimazole (10 mcg), Fluconazole (25 mcg), Amphotericin-B (20 mcg) and Nystatin (100 units) were tested. The zones measured only that is showing complete inhibition and the diameters of the zones recorded to the nearest millimeter.

Mutagenesis

Physical Mutagenesis

For the analysis of survival rates by UV (Ultra Violet) mutagenesis, cells were grown for 24 hours at 28°C on YPD (Yeast extract Peptone Dextrose) medium plate. The cells were collected by centrifugation and suspended in sterile water and this step was performed once again. To this, 1 ml was transferred to 9 ml of sterile water (10^-1) and serially diluted up to 10^-5. From each dilution, 100µl was placed on a plates containing SDA medium, then the plates were placed under a UV lamp at a distance of 35 cm and were irradiated for various periods of time. Following irradiation, the plates were incubated at 28°C. From the plates, the survival colonies were selected and again swabbed on MHA medium to determine the changes in their susceptibility pattern.

Chemical mutagenesis

For the analysis of survival rates by Ethyl Methane Sulfonate (EMS) mutagenesis, cells were inoculated into liquid YPD medium and grown for 24 hours at 28°C. The cells were washed once with sterile water, diluted and suspended in 1 ml of 0.1M sodium phosphate buffer (pH 7.0). To the suspension, 2% of EMS was added. After various incubation times, the cells were washed once with 1 ml of 5% sodium thiosulfate, suspended in water and spreaded on SDA plates and incubated at 28°C. The survived colonies from SDA plates were again swabbed on MHA plates to determine the changes in their susceptibility pattern.

RESULTS

The prevalence of Candida albicans among the low socio-economic group was assessed and recorded in Table 1. Totally 100 urine samples were collected from the low socio-economic group in Erode district. Among the 100 samples 26(26%) showed positive characteristics similar to Candida albicans.

Table 1: Prevalence of Candida albicans from low socio economic group

<table>
<thead>
<tr>
<th>Result</th>
<th>Prevalence of Candida albicans</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Positive</td>
<td>26 (26%)</td>
<td>26</td>
</tr>
<tr>
<td>Negative</td>
<td>74 (74%)</td>
<td>74</td>
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<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
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These isolates were identified and confirmed based on phenotypic diagnostic methods (the colour of colonies on HICHROME agar (Fig.1), germ tube formation, Chlamydomoses production on Corn meal agar medium and lack of pellicle formation on SDA broth).

Table 2 also shows the phenotypic characteristics of Candida tropicalis, Candida glabrata, Candida dublinsenis and Candida kruzei studied during the study period. Figure 1 shows the Candida albicans colonies (green) on Hichrome agar medium.

The In vitro antifungal activities of four azole derivatives (Itraconazole (IT) 10 mcg, Ketoconazole (KT) 10 mcg, Clotrimazole (CZ) 10 mcg and Fluconazole (FLC) 25mcg), Amphotericin-B (AP) 100 units and Nystatin (NS) 100 units against 26 clinical isolates of Candida albicans are given in Table 3.

Antifungal test results indicate that the yeast isolates were susceptible to Amphotericin-B 23(88.46%), Ketoconazole 9(34.62%), Fluconazole 5(19.23%) and Nystatin 2(7.69%). Various resistant levels were detected against other antifungal drugs but to Itraconazole and Clotrimazole all the isolates of Candida albicans were found to be 100% resistant. Two isolates of Candida albicans showing higher resistance patterns were selected and subjected to the physical (Ultra violet radiation (UV)) and chemical mutagenesis (Ethyl methyl sulfonate (EMS)).

Table 4 shows the surveillance rate of the isolates after mutagenesis. Even after the exposure of Candida albicans to physical and chemical mutagens they produced the colonies of growth on Muller-Hinton agar.

The UV and EMS exposed isolates of Candida albicans were tested with the same antifungal agents which are tested against the isolates before mutagenesis.
The urinary tract is colonized by both superficial and life-threatening Candida albicans, which are able to thrive and spread within the anatomical site most conducive to the development of infections [12]. Candida albicans is the predominant cause of invasive fungal infections and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization [13,14]. Several Candida species are commensal and colonize the skin and mucosal surfaces of humans. Critically ill or otherwise immunocompromised patients are more prone to develop both superficial and life-threatening Candida infections [15].

**DISCUSSION**

_Candida albicans_ is the predominant cause of invasive fungal infections [12] and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization [13,14]. Several _Candida_ species are commensal and colonize the skin and mucosal surfaces of humans. Critically ill or otherwise immunocompromised patients are more prone to develop both superficial and life-threatening _Candida_ infections [15].

_Candida albicans_ prefers the presence of moist areas of the body, which is why it is usually found in the genital and oral regions. Many other microorganisms grow in healthy mucous membranes in the body. However, when other organisms become depleted, the yeasts are able to outcompete for the limited resources. When the yeast takes over in the mouth or throat, it is often called ‘thrush’ and causes a sore throat along with a white coating in the mouth.

When the infection is in the female genital regions, it is called vulvovaginitis and can cause extreme itching and burning sensations along with possible white discharge. An infection caused by _Candida_ is termed candidiasis or candidosis. Mycoses caused by these fungi show a wide spectrum of clinical presentations and can be classified as superficial, as with cutaneous and mucosal infections, to deep, widespread and of high severity, as is the case with invasive candidiasis. Although this commensal organism normally colonizes mucosal surfaces in an asymptomatic manner, it can become one of the most significant causes of a disabling and lethal infection [16, 17]. In the early 1980s, fungi emerged as major causes of nosocomial infections, mainly affecting immunocompromised patients or those who were hospitalized for long periods due to serious underlying diseases [18].

_Candida_ species belonging to the microbiota of healthy individuals can be found scattered in the environment. It is believed that most people usually have a single strain of _Candida_ in different places in the body for a long period. However, some individuals have more than one strain or species at the same time, and this is commonly observed among hospitalized patients [19]. Moreover, the potential for _Candida_ species to become pathogenic should be appreciated. A crucial component of this versatility is the fact that these organisms survive as commensals in diverse and distinct anatomical sites, each with its particular environment. Although _Candida_ species can infect different anatomical sites of the human host, evidence exists that immune protection is site-specific for each type. Moreover, cutaneous candidiasis and vaginal infections are more likely to be associated with a phagocytic response involving neutrophils and mononuclear phagocytes [18]. The urinary tract is the anatomical site most conducive to the development of infections in hospitalized patients, although this remains a problem of questionable significance [20, 18].

In the present study the prevalence and its antifungal susceptibility of _Candida albicans_ isolated from urine sample among the low socio-
economic group was assessed and reported. Totally 100 urine samples were collected from the low socio-economic group in Erode district. There are several features essential for in vitro morphological transition of *C. albicans*. The earliest workers with *C. albicans* found that changes in the growth environment of *C. albicans* led to different morphologies. However, they were unable to discover any environmental “morphogen” that acts alone and under all conditions to provoke the growth of cells from any *C. albicans* isolate in only one morphological form. Some environmental factors that favour the filamentation in *C. albicans* are: temperature of 37-40°C, pH around 7.0 and an initial blastospore concentration not exceeding 10⁵/ml. Further, certain chemicals, such as N-acetylglucosamine, amino acids, biotin, sulfhydryl compounds, heme group, zinc, and serum, are required. The production of germ-tubes in serum has remained the method of choice for identifying *C. albicans* in clinical specimens. Although serum is excellent at promoting the yeast to mycelial conversion, unfortunately its chemical complexity makes it unsuitable for studying the molecular basis for inducing the hyphal form. In the present study among the 100 urine samples 24(26%) showed positive characteristics similar to *Candida albicans*. These isolates were identified and confirmed based on phenotypic diagnostic methods (the colour of colonies on HICROMO agar, germ tube formation, Chlamydospores production on Corn meal agar medium and lack of pellicle formation on SDA broth). In the current study along with Calbicans, Candida tropicalis, Candida glabrata, Candida dubliniensis and Candida krusei were also isolated from urine. In Chile, the prevalence of *C. albicans* has changed, and a progressive increase of non-albicans infection has been observed. *C. parapsilosis* was the most frequent species followed by *C. tropicalis* and *C. glabrata*. According to the Brazilian Candidaemia Study, *C. albicans* accounted for 40.9% of cases in Brazil, followed by *C. tropicalis* (20.9%), *C. parapsilosis* (20.5%) and *C. glabrata* (4.9%) [1,2,22]. In a study in United States which conducted by Richter, 593 cases of vaginal yeasts, including *C. albicans* (420 cases), *C. glabrata* (112 cases), *C. parapsilosis* (30 cases), *C. krusei* (12 cases), Saccharomyces cerevisiae (9 cases), *C. tropicalis* (8 cases), *C. lusitaniae* (1 cases) and Trichosporon (1 cases) were isolated [23]. In a study conducted in Turkey in 2000 on clinical isolated *Candida from vagina*, the most frequent species were *C. albicans* (50%), *C. glabrata* (26.90%), *C. krusei* (11.55%), *C. kefyr* (8.97 %), *C. tropicalis* (1.28 %) and *C. parapsilosis* (1.28 %), respectively [24]. In a study in Belgium in 2002, *C. albicans* was recognized as the most frequent pathogen with a frequency of 68.3%, followed by *C. glabrata* and *C. parapsilosis* [25]. In another study in the United States in 2005 which conducted by Trama et al., 1316 positive cultures including *C. albicans* (80.2 %), of *C. glabrata* (14.3 %), of *C. parapsilosis* (5.9 %) and of *C. tropicalis* (8%) were recognized [26]. According to another study in Bangladesh in 2007 from 172 RVVC patients 125 cases (72.7%) were *C. albicans*, 29 cases (16.9%) were *C. glabrata*, 13 cases (7.5%) were *C. tropicalis*, and about 5 cases (2.9 %) were *C. krusei* [27]. In 2009 a study was conducted on 1000 women, from 215 studied VVC patients in this study, the most frequent species were *C. albicans* (46.9 %), *C. glabrata* (36.7 %), *C. parapsilosis* (10.2 %), *C. tropicalis* (2.8 %), *C. kefyr* (1.9 %) and *C. krusei* (1.4 %) [28]. Based on a study conducted by Aqhami in Iran in 1386 on 128 women suffered from vaginal illnesses, *Calbicans* cover 83 % of isolated fungal species, the 17% remaining cases include *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* [29]. From the past studies by other authors and the current study also reveals the same result that *Calbicans* was the predominant strains noted in all the studies. Candiduria is a common nosocomial infection, which involves the urinary tract system as asymptomatic candiduria. The disease is most commonly caused by *Calbicans* [30]. In the current work 26 isolates of *Candida* was isolated from the urine sample of females belonging to low socio-economic group. In previous studies they isolated 22% of *Candida* species in urine samples from patients admitted to intensive care units [31].

Data from previous studies suggest that prolonged or intermittent treatment with antifungal agent modifies *Calbicans* prevalence, increasing the frequency of other *Candida* species. Acquired resistance has been associated with mutations in the target gene leading to lower affinity of the azole compound to the enzyme, upregulation of the enzyme level or by active transport of the azole out of the cell mediated by efflux pumps. Antifungal test results of the present study indicate that the *Calbicans* were susceptible to Amphotericin-B 23(88.46%), Ketoconazole 9(34.62%), Fluconazole 5(19.23%) and Nystatin 2(7.69%). Various resistant levels were detected against other antifungal drugs but to Itraconazole and Clotrimazole all the isolates of *Candida albicans* were found to be 100% resistant. In previous work they described about the conventional antifungal drugs and their cellular targets [32]. Among the azole chemical class described Ketoconazole, Fluconazole, Itraconazole target against Ergosterol synthesis, Amphotericin-B and Nystatin belonging to polycenes chemical class target against the Ergosterol (membrane function). Resistance may involve selected azoles or several azoles depending on the underlying mechanism and the various mechanisms may act alone or in concert [33, 34]. Although azole resistance has been described in invasive isolates, most resistant isolates have been detected after long-term treatment of mucosal infections. Overall, azole resistance in isolates belonging to normally susceptible species is still an infrequent event despite their use for several decades and nowadays for prophylaxis, empirical and preventative therapy as well as for the management of proven disease [35, 36]. However, a recent study reported reduced fluconazole susceptibility in 19% of 243 candidaemia cases including in 8% *C. albicans*, 4% *C. tropicalis* and 4% *C. parapsilosis*.

Mutations are changes to the genome of an organism. These are normally caused at low frequencies by spontaneous errors in DNA replication. The frequency of mutation is increased by mutagens, which include environmental and chemical agents such as ionizing radiation. UV irradiation, methyl-nitro-nitrosoguanidine, ethylmethane-sulfonate, nitrous acid and many other natural and artificial chemicals. Induced mutations can include changes not only to the nucleotide sequence, but also the structure and function of the DNA such as the creation of pyrimidine dimers and hydroxyl radicals. Different mutagenic agents have been used to obtain *Calbicans* mutants. In the current work two *Candida albicans* isolates which showed sensitivity to two different antifungals were selected and subjected to UV mutagenesis and chemical mutagenesis. The results revealed that both the isolates become resistant to all the antifungals, tested against the two strains of *Candida albicans* after mutagenesis experiments by both the physical (UV) and chemical method (EMS). The resistance of the isolates may be acquired due to the exposure of colonies to the physical and chemical mutagens.

CONCLUSIONS

Today, the rapidly evolving issue of resistance demands the obvious need for dissecting completely new regulatory mechanisms that could be targeted to control resistance. Novel mechanisms described earlier for pathogenic fungi clearly hold promise that can facilitate the development of new and exciting chemical strategies to efficiently control the human fungal diseases. Elucidating these mechanisms may provide new foundations for antifungal chemotherapy and can present an exciting challenge for the future investigation.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

REFERENCES


