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PREVALANCE OF CANDIDIASIS IN CHILDREN IN MUMBAI

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Abstract

This study aims to determine the prevalence of infection of Candida species and its resistance to anti-fungal agents amongst children in Mumbai. Total 169 clinical isolates of Candida species from various clinical specimens namely blood, urine, stool; Cerebral spinal fluid, liver abscess, Endotracheal secretion and ventric tip were included in the study. They were screened and identified by staining, culture and microscopy, followed by isolation on specialized chromogenic agar. Antifungal susceptibility tests of these isolates were carried out against Amphotericin B (50mcg), Itraconazole (10mcg), Clotrimazole (10mcg), Ketoconazole (30mcg), Miconazole (30mcg), and Fluconazole (10mcg) and were reported based on CLSI guidelines (2008). The prevalence of Candida infections was found to be greater in males (69%) as compared to female (31%) children. In the age group of 0-5 years, the occurrence of Candida infection was substantially higher as compared to the others age group. Candida albicans was found to be the major causative agent of fungal infections. The susceptibility of isolates to antifungal agents was found to differ in male and female patients. Overall, highest resistance was observed to Fluconazole (27.1%) while, lowest resistance to Amphotericin B (28.7%). Males exhibited highest resistance to Fluconazole (27.1%) while, females showed highest resistance to Amphotericin B (28.7%). Higher resistance was found to the azole group of antifungal agents. Hence, Amphotericin B can be the choice of treatment of Candida infection in children followed by Miconazole.

Keywords

Candida Albicans, Fluconazole, Amphotericin B

1. Introduction

The genus Candida belongs to yeast and is the most common cause of fungal infections worldwide. The genus is composed of a heterogeneous group of organisms. More than 150 species exists, of which 20 different Candida species are known to be etiological agents of human infection. However, more than 90% of invasive infections are caused by Candida albicans (C. albicans), Candida glabrata, Candida parapsilosis, Candidatropicalisand Candida krusei. (Bandi et al., 2014; Mohandas & Ballal, 2011; Pfaller & Diekema, 2007).

Candida is present in low number on healthy adult skin and C. albicans is part of the normal flora of the mucous membranes of the respiratory, gastrointestinal and female genital

tracts. Overgrowth of several species including C. albicans can cause superficial infections such as oropharyngeal candidiasis and vulvo vaginal candidiasis. Candidiasis is a yeast infection that is caused by fungal microorganism, most often the C. albicans. Candidiasis is also known as thrush and can cause yeast infections in many areas of the body .These commonly include the mouth, vagina and digestive tract.(Mohandas & Ballal, 2011; Sardi et al., 2013; Sharma & Solanki, 2014).

Immuno compromised patients are at a higher risk of developing infection because they lack the basic mechanisms of cellular defense. Majority of children, at one time or other suffer some or the other form of fungal infection. For instance, if a child develops a rash on the buttocks or white patches in the mouth, it is as a result of fungal or yeast infection. Fungal infections which were quite rare at the beginning of this century are now increasingly growing at a rapid rate. This is probably due to the result of the increase in number of immune compromised children. Fungi are very good at taking advantage of some abnormality in the human host and thus, virtually every fungal infection is opportunistic .The expanding population of immune-compromised patient that use intravenous catheters, total parenteral nutrition, invasive procedures and the increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of these infections (Sardi et al., 2013).
The aim of the study was to identify the spectrum of Candida species in clinical infections and to identify their resistance pattern to available antifungal agents. Identification of Candida at species level and their antifungal sensitivity testing would help us to achieve better clinical results and empirical treatment regimens and guiding clinical practice.

2. Materials and Methods

2.1 Isolation of Candida from Clinical Specimen

A total of 169 Candida isolates from various clinical specimens namely blood, urine, stool, Cerebral spinal fluid (CSF), liver abscess, Endotracheal (ET) secretion, ventric tip were included for the study. These isolates were mainly sent from B.J. Wadia hospital for children, Mumbai for speciation and antifungal testing. The study included the isolates collected from 16th May 2011 to 18th February 2015. Basic culture media used for isolation of Candida species was Sabouraud Dextrose Agar (SDA) or broth. Study was carried out in the Mycology section of Clinical Pathology Department of Haffkine Institute, Mumbai.

2.2 Identification of Isolates

Initially Isolates were screened for budding yeast like cells with the help of Gram staining and then cultured on SDA. The Candida isolates which were obtained were further speciated by the Germ tube test, culturing on chromogenic medium like HiCrome Candida Differential Agar (M1297A HiMedia Laboratories Pvt. Ltd.) This agar has chromogenic substances which helps in the rapid identification of the Candida species, based on the reaction between the specific enzymes of the different species and the chromogenic substance. As per the colour code provided with the chromogenic media, C. albicans produces light green colonies, Candida krusei produces purple fussy colonies, Candida glabrata produces creamy white colonies and Candida tropicalis produces blue metallic colonies. (Figure 1) The isolates were also identified on the basis of microscopic observation and Carbohydrate Assimilation test. In Carbohydrate Assimilation test, carbohydrate free yeast nitrogen base agar was used, on which different filter paper discs containing carbohydrate were placed. Also Carbohydrate fermentation test was conducted by using sugar fermentation media and observed for color change and gas production (Bhagat BP 2014; Patel, et al., 2012).

C.glabrata on

Figure 1: *Differentiation on Hi-chrome Agar*

2.3 Antifungal Susceptibility Testing

Antifungal susceptibility test was performed using disc diffusion method and interpreted using CLSI guidelines (2008) (formerly known as NCCLS). The antifungal drugs used were Amphotericin B (50mcg), Itraconazole (10mcg), Clotrimazole (10mcg), Ketoconazole (30mcg), Miconazole (30mcg), and Fluconazole (10mcg). Antifungal discs were procured from HiMedia Laboratories Pvt. Ltd Mumbai. (Klevay et al., 2005; Lozano et al., 1999; National Committee for Clinical Laboratory Standards, 2008).

3. Results

Table 3.1: *Frequency of Candida Species Isolated from Clinical Samples*

Sr. No	Sample	Percentage distribution
1	Blood	47%
2	Urine	37%
3	Stool	11.8%
4	CSF, ET secretion, Pus, Ventric tip and Liver abscess,	2.8%
5	Sputum	1.4%

Out of 169 Candida isolates collected, 47% isolates were grown from blood, 37% isolates were from Urine, 11.5% isolates were from Stool, 1.4 % from sputum and 2.8% isolates were from CSF, ET Secretion, pus, ventric tip, liver abscess [Table 3.1].

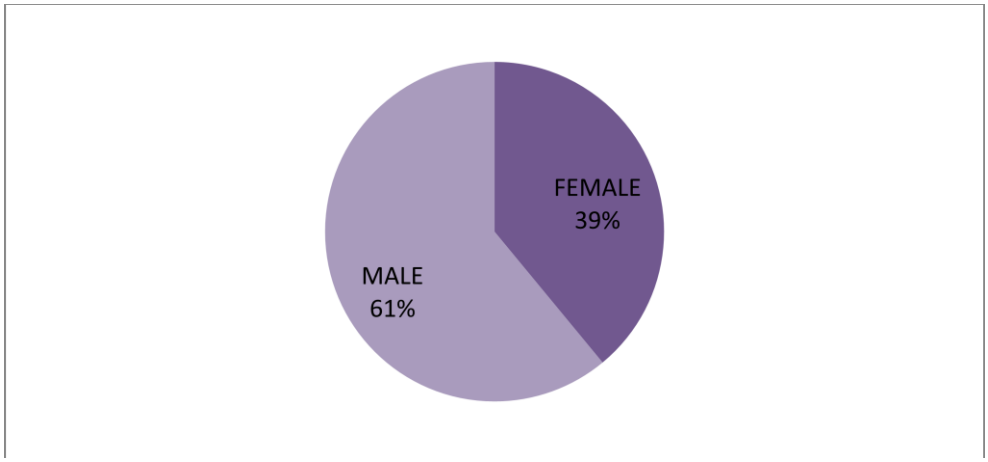

Figure 2: *Gender-wise susceptibilities to Candida infections*

Of the total number of isolates, 61% of isolates were obtained from male patients and 39% were from female patients. [Figure 2]

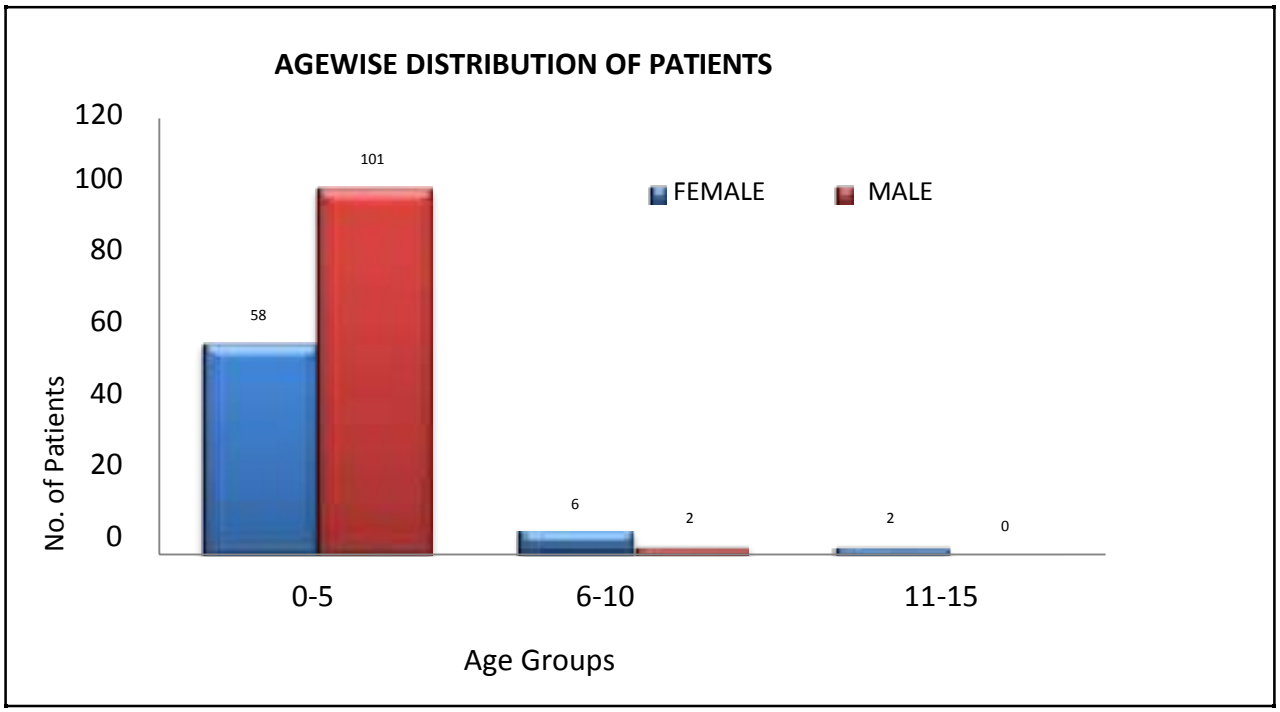

Figure 3: *Age-wise Distribution of Patients*

Patients included in this study were distributed in three groups, 1stgroup comprised of patient falling in the age group 0-5 years, 2nd group comprised of patient falling in age group 6-10 years, 3rdgroup comprised of patient in age group 11-15 years. It was found that 101clinical isolates of Candida isolated from male patients and 58 from female patients, were found to be in the age group of 0-5 years, whereas in the age group of 6-10 years, 06 isolates were from female patients and 02 were from male patients. Whereas, the age group 11-15 years had 02 isolates from female patients only. This implies fungal infections caused by opportunistic organisms like Candida mainly causes infection in young children, who havelesser immunity compared to higher aged children. [Figure 3]

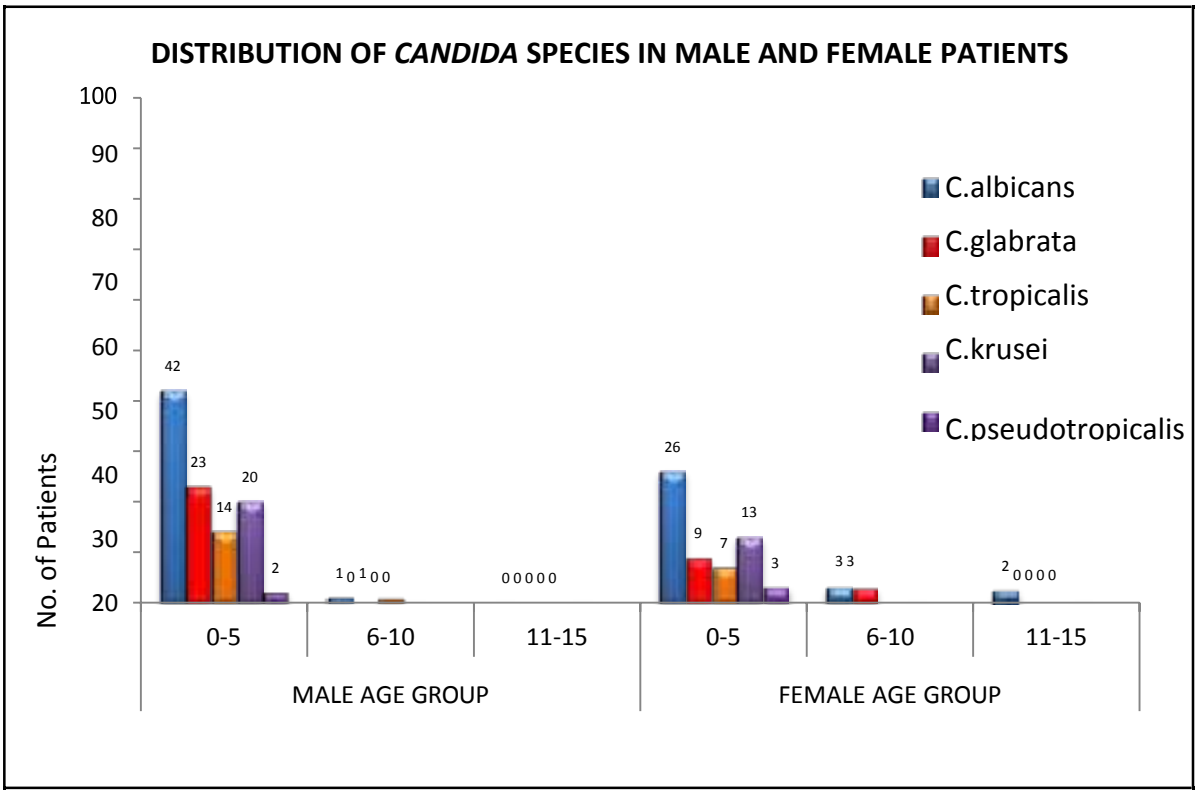

Figure 4: *Distribution of Candida Species in Male and Female patients*

Further it was observed that, in case of male patients, in 0-5 year's age group, C. albicans was major causative agent followed by Candida glabrata, Candida krusei, Candida tropicalis and Candida pseodutropicailis. In case of female patients, in 0-5 year's age group, C. albicans still remains the major causative agent but Candida krusei was the second major causative agent followed by Candida glabrata, Candida tropicalis and Candida pseodutropicalis. In age group 6-10 years and 11-15 years, the prevalence of infection was too low to determine any major causative agent. [Figure 4]

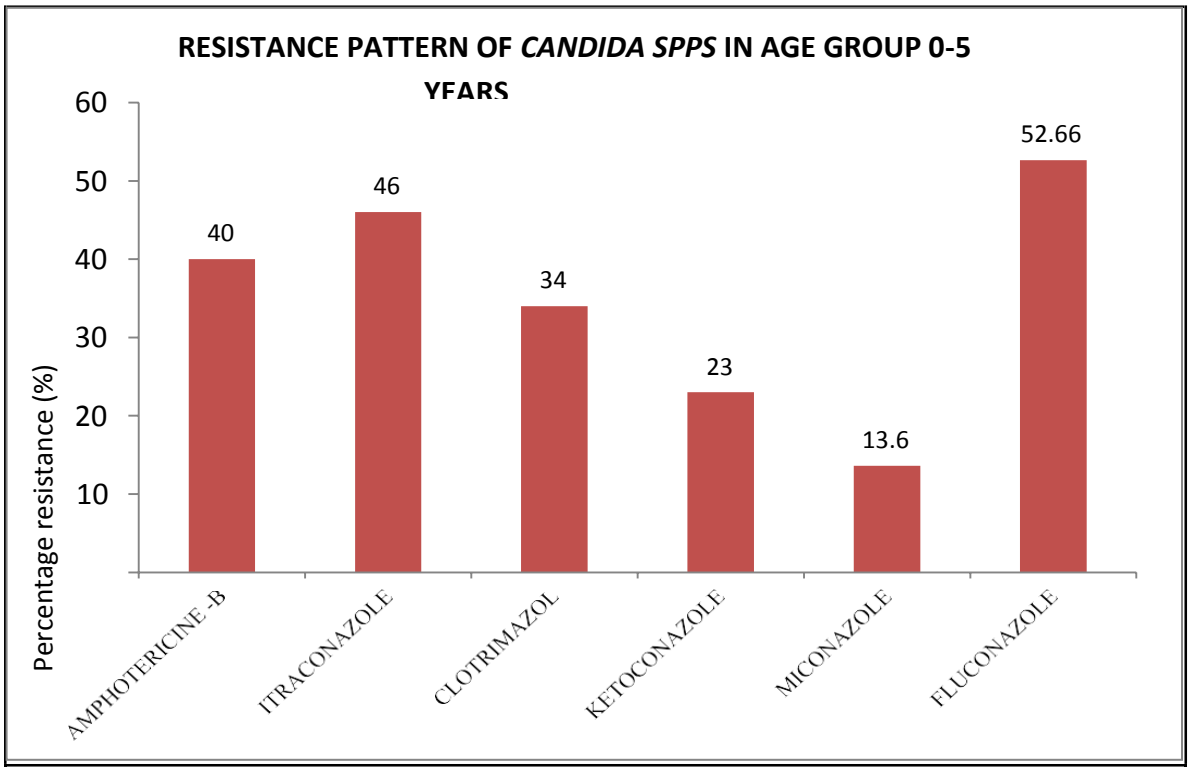

Figure 5: *Resistance pattern of Candida species*

Resistance pattern of Candida isolates from patients in age group 0-5 years indicated isolates were more resistant to azole group of drugs than Amphotericin B. In case of azole group resistance decreased from maximum in Fluconazole followed by Itraconazole, Clotrimazole, Ketoconazole and least to Miconazole. [Figure 5]

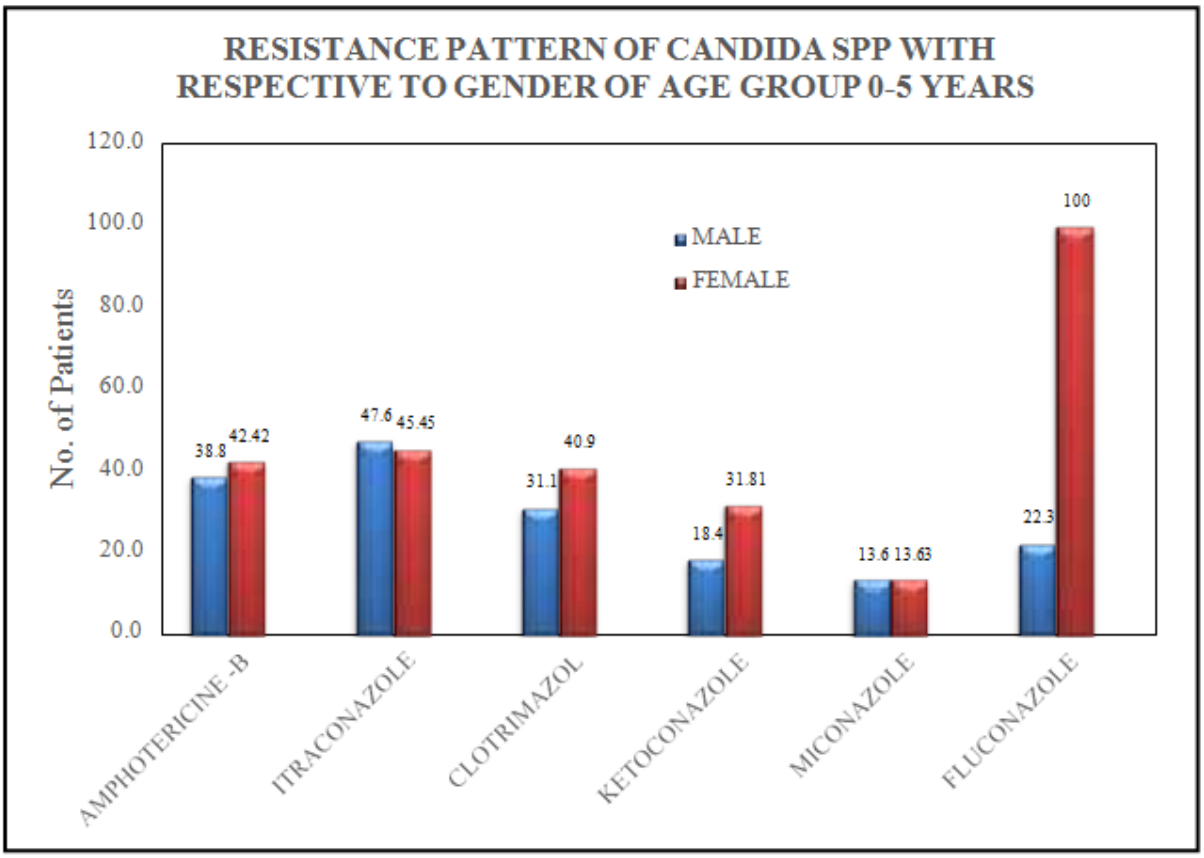

Figure 6: *Resistance pattern of Candida spp with respective to gender*

The antifungal sensitivity test of 169 Candida isolates highlighted that, 22.33% resistance was found to Fluconazole in case of males in the age group of 0-5 years followed by Itraconazole 47.57%, Amphotericin B 38.83%, Clotrimazole 31.06%, Ketoconazole 18.44% and Miconazole 13.59%. In case of females, maximum resistance was observed to Fluconazole 100% followed by Itraconazole 45.45%, Amphotericin B 42.42%, Clotrimazole 40.90% , Ketoconazole 31.81% and least to Miconazole 13.63%.[Figure 6]

4. Discussion

Amphotericin B, polyene, a fungicidal agent, has been standard treatment for Candida infection for decades. But toxicity of its conventional form and costs of its lipid form, limit its use. More recently, azole group of antifungal compounds, with lower Cytotoxicity and better

efficacies, have emerged as the principal drugs used in treatment of Candida infections. However, prolonged use of azole group drugs leads to maximum resistance as compared to

Amphotericin B. Hence Amphotericin B becomes the drug of choice for treatment of Candida infection.

Previous studies reported the susceptibility profile of Candida isolates. They found, 92% of isolates sensitive to Amphotericin B and 36% to Fluconazole (Kothari, 2008). The study from South India showed 92% isolates sensitive to Amphotericin Band 75% to Fluconazole. While some authors reported all of the tested yeasts were susceptible to Fluconazole and Amphotericin

B. Among the examined azoles, a high resistance rate in the isolated yeasts was found with Ketoconazole (Zomorodianet al, 2011).

In case of our study maximum resistance was observed against azole group of drugs. Hence Amphotericin B can be the drug of choice for treatment of Candida infections. But, in case of female patients, variable resistance to Amphotericin B is observed to C.albicans and Non-Candida albicans. This makes it important to determine the species of Candida isolates and its antifungal sensitivity before its treatment.

5. Conclusion

To conclude, the present study showed, prevalence of Candida infections in male patients is twice as compared to female patients. Prevalence of non-Candida albicans was found to be higher as compared to C.albicans. The age group division highlighted that, children between 0-5 years of age were at higher risk of developing Candidiasis. Speciation of Candida isolates on chrome agar medium led to rapid identification of candida species. Both C.albicans and non-C.albicans were found to be resistant to Azole group of drugs compared to Amphotericin B, but the resistance pattern to individual drug varied. The maximum resistance was found against Itraconazole followed by Fluconazole, Clotrimazole and Miconazole. Therefore, the choice of drug for treatment of candida infection can be Amphotericin B followed by Miconazole.

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