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Identification of *Candida Sp.* and their Antifungal sensitivity from patients at a tertiary care hospital

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Objective: In this analysis, we evaluated the prevalence of *Candida* Sp. in patients visiting the department of medicine and gynecology. Additionally, we assessed the known *Candida* sp.'s antibiotic sensitivity profiles.
Methods: From January 2011 to February 2012, this prospective, cross-sectional study was undertaken at the IMS and SUM hospital and included the infection-causing organisms. Specific traditional media techniques helped to confirm the existence of species difference. Using disc diffusion techniques, the antifungal susceptibility of distant Candida species has been assessed.
Results: E bulk of the 219 Candida isolates were from the departments of medicine (59; 26.9%) and gynaecology (78; 35.6%). Additionally, 75 (34.2%) of the samples were from married women, whereas 144 (65.8%) were from unmarried females. Candida glabrata 30(13.69%), Candida tropicalis 26(11.87%), Candida krusei 17(7.76%), Candida parapsilosis 12(5.47%), Candida dubliniensis 3(1.37%), and Candida lusitaniae 3(1.37) were the next most prevalent species after Candida albicans, which had 128 (58.45%) of the total. Amphotericin B has proven to be the least effective against all isolates, with a susceptibility charge of 213 (97.26%). When compared to fluconazole 32 (14.61%), voriconazole 40 (18.26%) showed the highest level of resistance.
Conclusion: Amphotericin B is the most effective antifungal for skin infections, according to this study. Candida species have shown resistant to numerous antifungals.

Keyword: *Candida* sp., *C. albicans*, antifungal sensitivity test, resistant, CHROMagar candida

Introduction

Fungal infections are a major clinical disease that significantly increases patient morbidity and death around the globe [1]. The pathogens that cause colonisation and infection are the natural commensals of the mouth cavity, GIT, and mucosal surfaces of the body [2]. With the rise in immunocompromised individuals, the incidence of fungus infections has gone up recently.Patients with cancer undergoing chemotherapy or radiation, those with diabetes [3], those using immune-suppressants, neutropenic patients [4], those receiving long-term steroids or antibiotics [5], and others commonly have Candida species identified from them [6]. It has been linked to septicaemia, respiratory infections, UTIs, mucocutaneous infections, and cutaneous infections. In critically sick patients in the ICU, invasive fungal infections frequently result in sepsis, severe sepsis, and septic shock, with Candida species being the most frequent cause of fungal sepsis, especially in the hospital acquired illnesses [7]. It is known that more than 17 distinct Candida species may cause infections in people. Even though Candida albicans is the fungal pathogen that is most frequently isolated from

clinical samples, non-albicans Candida species are gradually taking over as the main pathogens [6]. Furthermore, the growing use of anti-fungal medications for both treatment and prevention, especially in ICU patients, has resulted in the development of resistance against medications that are often used to treat fungal infections, such as different azoles [2, 4, 6]. The Candida species, however, exhibit varying levels of resistance to different antifungal medications. In order to effectively manage sepsis patients in our setting, this study was conducted to identify distinct Candida species from specimens of patients who had been clinically diagnosed with the condition and their antifungal susceptibility pattern. This study's goals included isolating and identifying the species of Candida from samples of oral and vaginal infections as well as evaluated the drug susceptibility of each species of Candida.

Materials and Methods

All of the patients who visited the otolaryngology and gynaecology departments over the course of a year participated in this prospective research. Therefore, samples were processed for fungal culture and inoculated on Sabourad's Dextrose Agar (SDA) if Gramme stained smears revealed the presence of any yeast cells or yeast-like cells with budding and with or without pseudohyphae. For 24 hours, plates were incubated aerobically at 37^0C. After a night of incubation, the colonies of Candida species were collected. Colony shape on SDA, colony colour on Candidal Differential Agar Media, germ tube test, and chlamydospore development were used to identify the colonies. Colour was used to distinguish the coloniesCandida albicans was identified using the colour of the colonies on HiCrome, a germ tube test, and observation of chlamydospore development on cornmeal agar. A well-isolated SDA colony was emulsified in 0.5 ml of human serum for the germ tube test using a sterilised straight wire. The test tubes were incubated for no more than two hours at 350C. On a clean, grease-free slide, a drop of the serum sample was deposited, and a cover slip was placed on top of it. The existence of germ tubes was next checked on this slide using a microscope's 10X and 40X objective lenses. A genuine germ tube is a filamentous extension of the yeast cell that does not constrict at the neck and *C. albicans*, respectively. According to CLSI standards for assessing anti-fungal drugs for yeasts, an antifungal susceptibility test for Candida species was performed.

Results

A total of 219 (7.28%) samples tested positive for candida infections, with 78 (35.62%) coming from the department of gynaecology and the remaining samples coming from the department of otolaryngology. All of these positive samples formed smooth, glossy, cream to white colonies that are typical of Candida species on the SDA. Gram-stained samples of these Candida-positive colonies were only processed for the germ tube (GT) test if the yeast cells contained budding were circular to oval and purple in colour. There were 88 (40.18%) strains that were GT negative and were classified as Candida species, whereas a total of 131 (59.82%) strains developed germ tubes and were thus classified as either *C. albicans* or C. dubliniensis. Utilising CHROM agar Candida and maize meal agar, species-level identification was carried out. *C. albicans* 128(58.45%) was the most prevalent species among all the positive isolates based on growth on both medium, followed by *C. glabrata* 30(13.69%), *C. tropicalis* 26(11.87%), *C. krusei* 17(7.76%), *C. parapsilosis* 12(5.47%), *C. dubliniensis* 3(1.37%), and C. lusitaniae 3(1.37). *C. glabrata* was the most prevalent species in NACs. Numerous biochemical assays were used to identify the Candida species, and the outcomes supported microscopic findings. In addition, 139 (63.5%) of the infections were picked up in hospitals as opposed to 80 (36.5%) that were picked up in the community. In both the OPD and the IPD, *C. albicans* was the most prevalent species, followed by *C. glabrata*, C. tropicalis, C. krusei, and *C. parapsilosis*. While other species were numerous in IPD, *C. krusei* was more common in OPD. Gynaecology has the highest incidence of Candida species (Table 1). It was shown that unmarried girls had higher concentrations of *C. albicans* and all NAC

species than married women. The three most common NAC species in single girls were C. tropicalis, *C. glabrata*, and C. krusei. For married women, *C. tropicalis* and *C. glabrata* were more prevalent than *C. albicans*. (Table-2). There were six age categories for the patients. Patients older than 60 years had the highest incidence of all Candida species, with *C. albicans* leading the pack, followed by *C. glabrata*, C. tropicalis, and C. krusei. *C. glabrata* and *C. tropicalis* were common in the age groups of 26–40 and 41–60. Most *C. krusei* was found in the group of people between the ages of 41 and 60. (Table-3). With a susceptibility rate of 213 (97.26%) in our investigation, amphotericin B was the most efficient antifungal against all Candida species. Three (2.34%) *C. albicans*, one (3.33%) *C. glabrata*, and one (5.88%) *C. krusei* species were shown to be resistant to amphotericin B. Curiously, fluconazole 32 (14.61%) and voriconazole 40 (18.26%) had the lowest resistance levels. The Candida species most resistant to fluconazole were *C. krusei* 4 (23.5%), followed by *C. albicans* 24 (18.75%), *C. glabrata* 3 (ten%), and *C. parapsilosis* 1 (8.3%). But *C. parapsilosis* had the highest level of voriconazole 4 resistance (33.3%), followed by *C. krusei* 4 (23.5%), *C. albicans* 26 (20.3%), *C. glabrata* 4 (13.3%), and *C. tropicalis* 2 (7.7%). For both azole antifungals, *C. dubliniensis* and C. lusitaniae showed a 100% susceptibility rate. (Table-4). Data from this study's antifungal resistance analysis revealed that 18 (or 8.2%) of the isolates had cross-resistance to both fluconazole and voriconazole. 16 (88.9%) of them were Candida albicans, and 2 (11.1%) were *C. glabrata*. Voriconazole and fluconazole were both cross-resistant to the *C. glabrata* isolates. One isolate of *C. albicans* was resistant to all three antifungals, i.e., amphotericin B, fluconazole, and voriconazole, whereas one isolate of *C. albicans* was resistant to all three. Of the *C. albicans* isolates, 14 (87.5%) were cross-resistance to fluconazole and voriconazole.

Table 1: Candida Species isolated from the study

C. albicans	*C. glabrata*	*C. tropicals*	*C. krusel*	*C. parapsllosis*	*C. dubllnieniensis*	*C. lusitaniae*	**Total**
Forty-seven	Nine	Ten	Five	Seven	Seven	zero	78 (35.6%)
Thirty-one	Nine	sex	Seven	Three	Three	One	59(26.9%)
Twenty	Six	Four	One	One	One	Zero	32(14.6%)
Thirteen	One	Three	Two	Zero	Zero	Zero	20(9.1%)
Five	Two	Zero	Two	Zero	Zero	One	10(4.6%)
Five	One	Three	Zero	Zero	Zero	One	10(4.6%)
Severn	Two	Zero	Zero	One	One	Zero	10(4.6%)
Forty three	Eleven	Eleven	Ten	four	Four	Zero	80(63.5%)
Eighty five	Nineteen	Fifteen	Seven	Eight	Eight	Three	139(14.6%
One twenty eight	Thirty	Twenty six	Seventeen	Twelve	Twelve	Three	219(100%)

Table 2: Gender wise distribution of Candida albicans and Non albicans (Nacs) species

Gender	**C.Albicans (128)**	**C.Glabrata (30)**	**C.tropicals (26)**	**C.krusel (17)**	**C.parapsllosis (12)**	**C.dubllnieniensis (3)**	**C.lusitaniae (3)**	**Total**	**Total isolates**
Unmarried girls	Eighty one	Twelve one	Seventeen	Twelve	Eight	Three	Two	Sixty three	144(65)%
Married women	Forty seven	Nine	Nine	Five	Four	Zero	One	Twenty eight	75(34.2%)

Table 3: Age wise Candida species distribution.

Gender	**Children (0-1)**	**Teenagers (12-18)**	**Young adults (19-25)**	**Age group Adults (26-40)**	**Middle-aged (41-60)**	**Senior citizen (>60)**	**Total**
Unmarried girls	four	Two	Thirteen	Forty-two	Thirty-five	Forty eighty	144(65.8%)

Married women	three	Two	Three	Nine	Twenty	Thirty-eight	75(34.2%)
Total	seven	four	Sixteen	Fifty one	Fifty-five	Eighty six	219(100%)

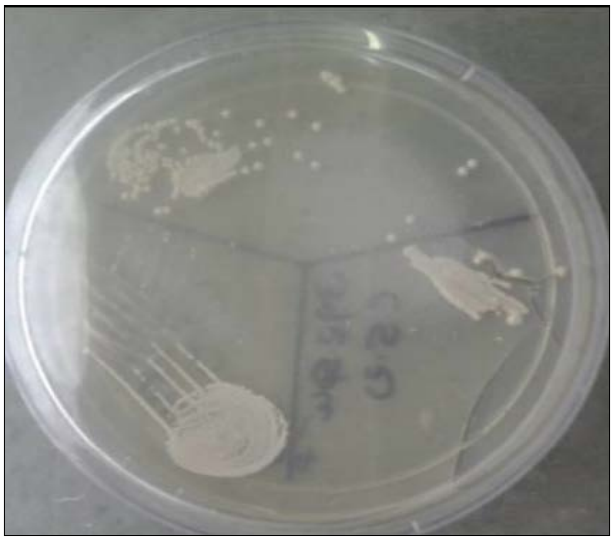

Fig 1: Isolation of Candida Species in SDA agar plate

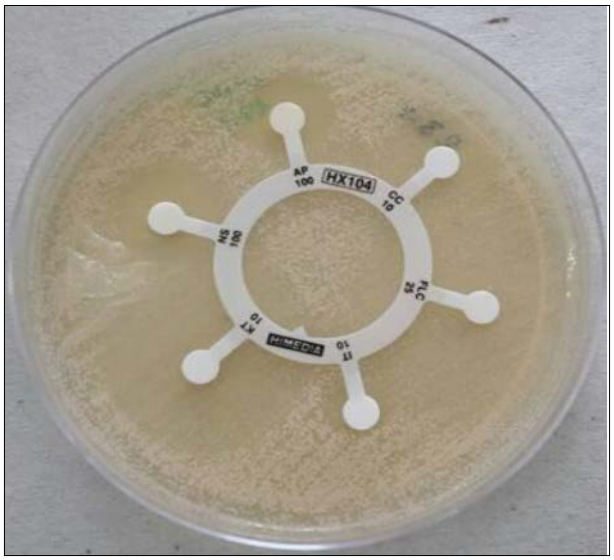

Fig 2: Disc diffusion methods for Antifungal resistance patterns of Candida species

Discussion

Since the choice of therapy is directly influenced by the virulence factors and antifungal susceptibility profile of *C. albicans* and NACs, accurate and speedy species identification is essential [7]. Similar to prior publications, *C. albicans* (58.4%) was the predominant pathogen in our investigation when compared to NACs [8-11]. Nucci *et al.* [12] also noted that *C. albicans* (37.6%) and *C. parapsilosis* and *C. tropicalis* were the primary causes of Candida infection. In our investigation, *C. glabrata* (13.7%), *C. tropicalis* (11.9%), *C. krusei* (7.8%), *C. parapsilosis* (5.5%), *C. dubliniensis* (1.4%), and *C. usitaniae* (1.4%) had the highest incidence of NACs. The most prevalent species of *C. glabrata* among NACs in clinical samples is an important finding of our investigation. Due to the significant prevalence of this species' enhanced resistance to commonly used antifungal medicines, this might be a disturbing concern. Patel *et al.* [10] Similar to our investigation, which

found that the largest percentage of Candida isolates were found in the urine and sputum (78, 35.6%, 59, 26.9%, and 32, 14.6%, respectively). According to a distinct epidemiological trend documented by Farooqi *et al*.13, C. tropicalis, *C. parapsilosis*, and *C. glabrata* were the most prevalent organisms. In our study, unmarried girls made up 144 (65.6%) more of the sample than married women made up 30.9%, which is consistent with Nardin *et al*. findings [14]. The fact that Candida species have a receptor for unmarried females' reproductive hormones is the cause of their high prevalence and virulence. Rashwas *et al*. [15] detected candiduria in 34.4% of teen females who are not married and 14.9% of married women. Aslam *et al*. [16] Nosocomial candidiasis was also shown to be more common in unmarried girls patients (56%) than in married women patients (44%). According to our findings, a significant proportion of single females seeking care at the QIH may be related to issues with personal hygiene. According to research by Furnaleto *et al*.17 and Al- Hussaini, the age group of >60 years and middle aged-group in our study had the highest prevalence of Candida infection [18].

In the present study, Candida infection rate was high in Gynaecologywards. However, other studies reported that Candida infection was more common in ICU and surgical ward [19]. With the exception of Candida albicans, Candida glabrata, and Candida krusei, Amphotericin B was shown to be extremely efficient against all tested species, which is consistent with the findings of De Almeida d *et al*. [20] This study's findings on antifungal susceptibility also showed a notable increase in azole resistance in NACs compared to *C. albicans*. The most resilient isolate was C. krusei, followed by *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. glabrata*. Oberoi *et al*. [21] *C. tropicalis* was shown to be very sensitive to fluconazole, whereas *C. glabrata* was highly resistant and *C. parapsilosis* was less resistant. In contrast to *C. parapsilosis* and *C. glabrata*, all local isolates of *C. tropicalis* that had been investigated exhibited fluconazole sensitivity. Badiee and Alborzi [22] report a *C. albicans* fluconazole susceptibility of 89.5%, which is fairly close to our findings. Resistance to fluconazole was 18.8%, comparable to the Sojakova *et al*., [23] Results revealed 227 Candida isolates have a 13% fluconazole resistance. Fluconazole resistance in NACs and *C. albicans* has alarmingly grown, according to research by Kaya *et al*. (68.7%). (63.2%) [24].

Conflict of Interest: Nil

Funding source: Nil

Ethical Clearance: This study was approved from the competent authority of our Institutional ethics committee.

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