INTRODUCTION

Importance of an epidemiological monitoring of yeasts involved in pathogenic processes is unquestionable due to the increase in trend of infections caused by various species of Candida over the last decade; so are the changes observed in species causing Candidiasis and empirical antifungal treatment.1 There is a rise in non-albicans Candida species being more prevalent with varied antifungal susceptibility. There is need for continuous surveillance of prevailing species and to know their antifungal susceptibility so as to aid in treatment of affected patients. The present study is taken to know the prevalent species isolated from various clinical specimens and their antifungal susceptibility testing by disc diffusion technique against various antifungal agents such as fluconazole, ketoconazole, voriconazole and itraconazole. C. albicans is the common and major pathogen considered to cause Candidiasis among Candida species, more frequently the immunocompromised individuals are affected suffering from HIV, using long-term immunosuppressive therapy in

Speciation and antifungal susceptibility testing of candida species isolated from clinical samples

Lokjan Singh¹, Sheetal U Harakuni², Bibek Basnet³, Keshab Parajuli⁴

¹Post Graduate Student, ²Professor, Department of Microbiology, KLE Academy of Higher Education and Research, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, ³Post Graduate Student, Department of Biochemistry, KLE Academy of Higher Education and Research, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, ⁴Lecturer, Department of Public Health, Asian College for Advance Studies, Purbanchal University, Nepal

ABSTRACT

Background: The importance of epidemiological monitoring of yeasts involved in pathogenic processes is unquestionable due to the increase in trend of infections caused by various species of Candida over the last decade; so are the changes observed in species causing Candidiasis and empirical antifungal treatment. Aims and Objective: To speciate the clinically isolated Candida species by phenotypic methods and to estimate the antifungal susceptibility of the isolated species against fluconazole, ketoconazole, voriconazole, itraconazole by disc diffusion method. Materials and Methods: A cross-sectional study conducted in 2018 in the Department of Microbiology, J. N. Medical College, KAHER, Belagavi Karnataka. Ethical Clarence was obtained from institutional ethical committee J.N. Medical College. Results: Out of 59 Candida isolates, Candida tropicalis was the predominant species 41 (69.49%), followed by Candida glabrata 5 (8.47%), Candida parapsilosis and Candida lusitaniae 4 (6.78%) respectively, Candida guilliermondii and Candida kefyr 2 (3.39%) respectively and the least one was Candida krusei 1 (1.69%). Voriconazole showed the highest level of sensitivity whereas Itraconazole has shown the least sensitivity pattern by disk diffusion method. Out of 59 Candida species, 52 (88.13%) were sensitive to Voriconazole, 44 (74.57%) were sensitive to Fluconazole, 40 (67.79%) were sensitive to Ketoconazole and the least sensitivity was shown by Itraconazole 30 (50.84%). Candida krusei and Candida guilliermondii showed 100% sensitive to Fluconazole, Voriconazole, Ketoconazole and Itraconazole respectively. Conclusion: Non-albicans Candida species are being common isolates from cases of candidiasis. Candida tropicalis is the predominant isolate, followed by Candida glabrata, Candida lusitaniae, Candida parapsilosis, Candida guilliermondii, Candida kefyr and Candida krusei. Most of the isolates were sensitive to Voriconazole.

Key words: Disk diffusion method; NAC; Sensitive; Predominant species

Address for Correspondence:
Lokjan Singh, Post Graduate Student, Department of Microbiology, KLE Academy of Higher Education and Research, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India. Mobile: +977-9842393339. E-mail: slokjan@gmail.com

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cancer and organ transplant patients, Azole resistance were seen more commonly in non-albicans Candida than the Candida albicans, therefore, antifungal susceptibility testing will help to choose the empirical antifungal treatment and to identify to species level; it will also help the epidemiology of Candida species particularly the source and mode of transmission so that we can prevent and control the transmission and resistant pathogens. Over the past 2-3 decades increasing numbers of incidence due to non-albicans Candida species is significant although considered as normal flora of human beings at the different sites such as skin, female genital tract, gastrointestinal tract, respiratory tract etc. Due to variety of predisposing factor it alters the host immune status of the individuals which lead to invasive Candidiasis. Virulence factors of Candida species plays as essential role to facilitate the pathogenesis of Candidiasis such as: Adhesion: that interact with the surface of endothelial and epithelial cells. Enzymes: Phospholipase, Esterase, Proteases, Lipase plays an important role to initiate the infection. Toxins: These are like endotoxin in bacteria which induce the anaphylactic shock, Virulent to the patients such as-glycoproteins. Phenotyping Switching: In which the organisms has the ability to grow in various morphological features such as unicellular budding (blastospore) to filaments pseudo hyphae and sometimes true hyphae which helps the organisms to survive in the host at different conditions or temperature to facilitate the pathogenicity.

MATERIALS AND METHODS

A cross sectional study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi. In the year 2018 from January 2018 to December 2018, isolates were collected from clinical samples by Universal sampling procedurewhere all the isolates from patients with Invasive Candidiasis, Suspected cases of Candidiasis were included.

Once the isolates of Gram positive yeasts were identified in Candidiasis such as;

Toxins: These are like endotoxin in bacteria which induce the anaphylactic shock, Virulent to the patients such as-glycoproteins.

Phenotyping Switching: In which the organisms has the ability to grow in various morphological features such as unicellular budding (blastospore) to filaments pseudo hyphae and sometimes true hyphae which helps the organisms to survive in the host at different conditions or temperature to facilitate the pathogenicity.

RESULTS

In Graph 1, among 59 Candida isolates Candida tropicalis was the predominant species 41 (69.49%), followed by Candida glabrata 5 (8.47%), Candida parapsilosis and Candida lusitaniae 4 (6.78%) respectively, Candida guilliermondii and Candida kefyr 2 (3.39%) respectively and the least one C. krusei 1 (1.69%).

In Table 1, our study out of 59 clinical samples sputum was 28.81% followed by vaginal swab 27.11% and the least collected samples cervical swab, Broncho-alveolar lavage and stool were 3.38% followed by Sub hepatic fluid, oral tissue and skin scraping were 1.69%.

In Tables 2 and 3, our study Voriconazole showed the highest level of sensitivity whereas Itraconazole had shown the least sensitivity pattern by disk diffusion method. Among all the isolated 59 Candida species, 52 (88.13%) were sensitive to Voriconazole, 44 (74.57%) were sensitive to Fluconazole, 40 (67.79%) were sensitive to Ketoconazole and the least sensitivity was shown by Itraconazole 30 (50.84%). Among 41 (69.49%) isolates of Candida tropicalis 37 (90.24%) were sensitive to Voriconazole, 31 (75.61%) were sensitive to Fluconazole, 28 (68.29%) were sensitive to Ketoconazole and 22 (53.22 %) were sensitive to Itraconazole. Candida krusei and Candida guilliermondii

![Distribution of isolates in percentage](image-url)

Graph 1: Distribution of isolates in percentage
showed 100% sensitive to Fluconazole, Voriconazole, Ketoconazole and Itraconazole respectively.

DISCUSSION

A total of 59 isolates were processed from the samples received in the Department of Microbiology of Jawaharlal Nehru Medical College, KAHER University, Belagavi. Among isolates Candida tropicalis was found to be the most predominant species whereas Candida krusei is the least to be isolated. In our study Candida tropicalis being the most predominant species 41(69.49%). Similar studies done by Bhawana et al,17 Singh R et al87 have reported Candida tropicalis, ranging between 26-59%, being the major isolate.3,14,17

A study carried out by Khadka. S et al18 2014-15 Nepal has found that Candida tropicalis is the major isolate among non-albicans Candida followed by Candida glabrata which is similar to our study.21 Various study in the past has shown the increasing numbers of non-albicans Candida species were

![Figure 1: Sensitive to Fluconazole, Voriconazole, Ketoconazole, Resistant to Itraconazole](image1)

![Figure 2: Susceptible to Fluconazole, Voriconazole, Ketoconazole, Itraconazole](image2)

| S.no. | Clinical specimens | Numbers | Percentage (%) |
|-------|--------------------|---------|----------------|
| 1     | Sputum             | 17      | 28.81          |
| 2     | Vaginal swab       | 16      | 27.11          |
| 3     | Urine              | 9       | 15.25          |
| 4     | Wound swab         | 4       | 6.67           |
| 5     | Long line tip      | 3       | 5.08           |
| 6     | Cervical swab      | 2       | 3.38           |
| 7     | Broncho-alveolar lavage | 2 | 3.38 |
| 8     | Stool              | 2       | 3.38           |
| 9     | Sub hepatic fluid  | 1       | 1.69           |
| 10    | Oral tissue        | 1       | 1.69           |
| 11    | Skin scrapping     | 1       | 1.69           |
| 12    | Nail clipping      | 1       | 1.69           |
| Total |                    | 59      | 100%           |

| Species of candida       | Fluconazole | Voriconazole | Ketoconazole | Itraconazole |
|--------------------------|-------------|--------------|--------------|--------------|
| C. tropicalis (n=41)     | 31          | 37           | 28           | 22           |
|                          | 75.61%      | 90.24%       | 68.29%       | 53.66%       |
| C. glabrata (n=5)        | 3           | 3            | 2            | 1            |
|                          | 60%         | 60%          | 40%          | 20%          |
| C. parapsilosis (n=4)    | 4           | 4            | 3            | 2            |
|                          | 100%        | 100%         | 75%          | 50%          |
| C. lusitaniae (n=4)      | 2           | 3            | 3            | 2            |
|                          | 50%         | 75%          | 75%          | 50%          |
| C. guilliermondii (n=2)  | 2           | 2            | 2            | 2            |
|                          | 100%        | 100%         | 100%         | 100%         |
| C. kefyr (n=2)           | 1           | 2            | 1            | 0            |
|                          | 50%         | 100%         | 50%          | 0.00%        |
| C. krusei (n=1)          | 1           | 1            | 1            | 1            |
|                          | 100%        | 100%         | 100%         | 100%         |
| TOTAL                    | 44          | 52           | 40           | 30           |
| PRECENTAGE               | 74.57%      | 88.13%       | 67.79%       | 50.84%       |
isolated which is comparable to our study, moreover these findings suggest that the non-albicans Candida species taking over Candida albicans day by day as an important pathogen since past 2-3 decades. Non albicans Candida has got direct impact on choosing the empirical antifungal treatment.18

Other non-albicans Candida isolated in our study were, Candida glabrata, Candida lusitaniae, Candida parapsilosis, Candida guilliermondii, Candida kefyr, Candida krusei. It is proved in many studies that distribution of Candida species follows geographic distribution. Thus our study adds-up to the observation of, Candida tropicalis, being prevalent in causing Candidiasis, in Eastern Asia.

In our study, isolates showed highest sensitivity to Voriconazole whereas were least sensitive to Itraconazole by disk diffusion method. Of all the isolated Candida species, sensitivity for Voriconazole was 88.13% (52) followed by Fluconazole (74.57%), ketoconazole (67.79%) and the least sensitivity was against Itraconazole 30(50.84%). Candida guilliermondii and Candida krusei, both being single isolates were sensitive to all the tested antifungals.

In a study done by Jangla et al9 Candida tropicalis and Candida parapsilosis were 100% sensitive to Fluconazole and Voriconazole. Sebastian. A et al20 have shown 62.2%, 58.8%, 47% sensitivity against Fluconazole, Itraconazole, Ketoconazole respectively which is nearly similar to that our study which shows 74.57% to fluconazole, 67.79% to Ketoconazole, 50.84% to Itraconazole respectively.9,20

Our study showed Voriconazole is an effective drug 52(88.13%) where as 100% sensitive to Voriconazole was found in a similar study done by DeepthiT et al23 60% by Dharwad. S et al,22 83% by Shafi FT et al25 respectively.21-23 Though Voriconazole is found to be effective in the study, the observation has to be practiced with a guard. The results need to be validated by another study where in clinical correlation would be appropriate.

CONCLUSION

Candida non-albicans species are being common isolates from cases of Candidiasis. Candida tropicalis is the predominant isolate, followed by Candida glabrata, Candida lusitaniae, Candida parapsilosis, Candida guilliermondii, Candida kefyr and Candida krusei. Most of the isolates were sensitive to Voriconazole and resistant to Itraconazole. Resistance was most commonly seen in Candida glabrata, Candida kefyr had shown 100% resistant against Itraconazole. Thus the study emphasizes the need for speciation of Candida isolates and recommends antifungal susceptibility for all the clinical isolates. To identify various isolates of Candida to their species level along antifungal sensitivity pattern is clinically significant for the appropriate choice of antifungal therapy.

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ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of Jawaharlal Nehru Medical College, KLE Academy Higher Education and Research, Karnataka, India.

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Authors Contribution:
LS- Concept and design of the study, data collection, analyzed and interpreted, reviewed the literature, manuscript preparation and critical revision of the manuscript; SUH- Concept, and review of literature and helped in preparing first draft of manuscript; BB- Prepared first draft of manuscript and critical revision of the manuscript; KP- Conceptualized study, literature search, statistically analyzed and interpreted, prepared first, draft of manuscript and critical revision of the manuscript and data collection.

Work attributed to:
Department of Microbiology, KLE Academy of Higher Education and Research, Jawaharlal Nehru Medical College, Belagavi, Karnataka, India.

Orcid ID:
Lokjan Singh - https://orcid.org/0000-0002-3293-3900

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