

Original Research Article

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## A Study on Identification and Antifungal Susceptibility Pattern of different *Candida* Species Isolated from Various Clinical Specimens in a Tertiary care Hospital of West Bengal, India

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### ABSTRACT

Candidiasis is the 4<sup>th</sup> most common organisms causing blood-stream infection. It is mainly caused by *Candida albicans* but non-albicans *Candida* such as *Candida tropicalis*, *Candida glabrata*, *Candida krusei* etc has also increased in last few years. These emerging pathogenic non-albicans *Candida* are developing resistance to most of the conventional antifungal drugs. To find out the different *Candida* species in various clinical specimens and determine their antifungal susceptibility pattern. All the *Candida* isolates recovered from various clinical samples during the period from August 2015 to August 2016 were included in this study. The presumptive diagnosis was done on the basis of wet mount, gram's stain, culture on Sabouraud's dextrose agar media. Once the colonies were confirmed speciation was done by germ tube test, corn meal agar inoculation, sugar assimilation test and CHROMagar *Candida* inoculation. Antifungal susceptibility pattern was carried out by disc diffusion method recommended by CLSI. The antifungal agents were fluconazole (10µg), amphotericin B (10µg), ketoconazole (10 µg) and nystatin (10µg). In the present study, a total of 302 various clinical specimens were collected. Among them 126 (41.7%) *Candida* isolates were found. The maximum number of *Candida* isolates were obtained from vaginal swab (42) followed by sputum (28) and oral swab (18). The most commonly found *Candida* isolates were *Candida albicans* (52.4%) followed by *C. tropicalis* (27%), *C. Krusei* (14.3%), *C. glabrata* (6.3%). All the isolates of *C. albicans* and *C. tropicalis* were sensitive to amphotericin B. The next most effective antifungal drug was ketoconazole with 85.7% (108). Fluconazole was the least effective drug in our study with sensitivity of 38% (48). *Candida* is one of the most commonly found nosocomial pathogens. The accurate species identification of *Candida* is important for treatment as Non-albicans *Candida* are resistant to most azole group of drugs and also because of rising anti-fungal resistance among different *Candida* species.

#### Keywords

Candidiasis,  
*Candida*,  
non-albicans  
antifungal  
agents.

#### Article Info

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## Introduction

*Candida* is yeast like fungus which causes commonest fungal disease (called Candidiasis) in humans affecting skin, mucosa, nails and various internal organs. It is caused by various species belonging to genus *Candida* with *Candida albicans* as the representative species (Chander, 2009). However in recent years non-albicans *Candida* are increasingly found in various clinical scenarios (Gullo, 2009). The infection may be acute or chronic, superficial or deep and its clinical spectrum is wide. It is found mainly as secondary infection individuals with some underlying immunocompromised conditions such as prolonged usage of antibiotics, chemotherapy, immunosuppressive drugs, long term catheterization etc.

Species identification of *Candida* is very much important especially for treatment purposes as not all species respond to the same treatment because of the development of antifungal resistance (Shivanand *et al.*, 2011). So the objective of this present study is *Candida* species identification among various clinical specimens and to determine their antifungal drug susceptibility.

## Materials and Methods

The present study was conducted in the department of Microbiology, in a peripheral tertiary medical college of west Bengal for a period of one year i.e. from August 2015 to August 2016. All the clinical samples from those patients having suspected fungal infections submitted in the microbiology department were included in this study. Samples were collected from blood, urine, sputum, oral swab, vaginal swab, pus, skin scrapping and nail scrapping. The preliminary presumptive diagnosis was made on the basis of wet mount, gram's stain, culture on SDA (Sabouraud's dextrose

agar) media. (Figure-1) Isolates other than *Candida* species were excluded from the study. Colonies were identified and confirmed by colony character and Gram's stain. Once the colonies were confirmed *Candida* species identification was done by germ tube test, corn meal agar inoculation, sugar assimilation test and CHROMagar *Candida* inoculation. Antifungal drug susceptibility testing was done by disc diffusion method on Muller Hinton Agar recommended by CLSI (CLSI, 2016). The antifungal discs used were fluconazole (10µg), amphotericin B (10µg), ketoconazole (10 µg) and nystatin (10µg).

The study proposal along with other relevant documents was submitted to the 'Institutional Ethics Committee' for review and approval. The study was commenced only after approval is obtained from appropriate authority.

## Statistical Analysis

Data were coded and entered into MS-Excel sheet. Statistical analysis were done using software SPSS 20 version. Descriptive and inferential statistics were used. Data were presented in percentages.

## Results and Discussion

In the present study, a total of 302 various clinical specimens were collected. Among them 126 (41.7%) *Candida* isolates were found. The maximum number of *Candida* isolates were obtained from vaginal swab (42) followed by sputum (28) and oral swab (18). This distribution is displayed in Table-1.

The most commonly found *Candida* isolates were *Candida albicans* (66) followed by *C. tropicalis* (34), *C. Krusei* (18), *C. glabrata* (08). The percentage wise distribution of different *Candida* species is shown in pie diagram-2.

All the isolates of *C.albicans* and *C.tropicalis* were sensitive to amphotericin B. The next most effective antifungal drug was ketoconazole with 85.7% (108). Fluconazole was the least effective drug in our study with sensitivity of 38% (48). Antifungal sensitivity pattern of *Candida* isolates is shown in Table-2.

The frequency of diseases caused by *Candida* has amplified over the last few years especially in immunocompromised patients. There is also an increased frequency of non-albicans *candida* commonly *Candida tropicalis*, *Candida glabrata*, *Candida krusei*. Our study shows occurrence of *Candida* infection was 41.7% in various clinical specimens. This finding is similar with the study done by Mohandas *et al.*, (2011).

In our present study, highest numbers of isolates (52.40%) were *Candida albicans*

followed by *C. tropicalis* (27%), *C. krusei* (14.30%) and *C. glabrata* (6.30%). These findings also correlates with Mohandas *et al.*, (2011) study.

In this study, speciation of *Candida* was done by germ tube test, corn meal agar inoculation, sugar assimilation test and CHROMagar *Candida* inoculation. Chromogenic agar media is a differential medium used in mycology laboratory for rapid identification of *Candida spp.* in comparison to conventional methods. It contains enzymatic substrates linked to different chromogenic compounds. When a specific substrate breaks down the substrate, the chromogenic substrate produce colours. The different colour variation is useful for the rapid and presumptive identification of *Candida* (Odds *et al.*, 1994). Consequently this will help for the early initiation of appropriate therapy.

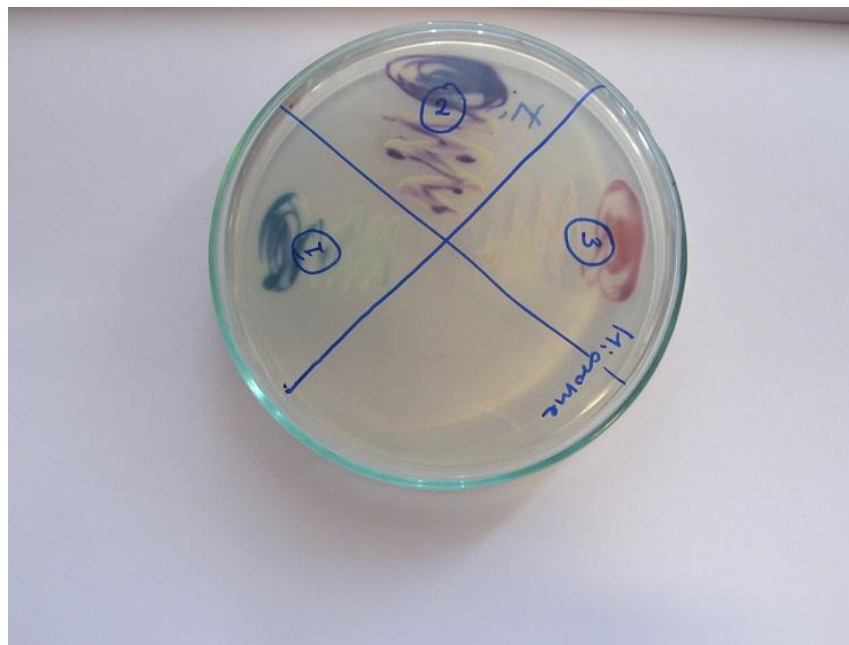
**Table.1** Distribution of *Candida* isolates according to various clinical specimens:

| Specimen         | Number of isolates | Percentage |
|------------------|--------------------|------------|
| Vaginal swab     | 42                 | 33.3%      |
| Sputum           | 28                 | 22.2%      |
| Oral swab        | 18                 | 14.3%      |
| Blood            | 06                 | 4.8%       |
| Pus & wound swab | 08                 | 6.3%       |
| Urine            | 12                 | 9.5%       |
| Nail scraping    | 05                 | 4.0%       |
| Skin scrapings   | 07                 | 5.6%       |

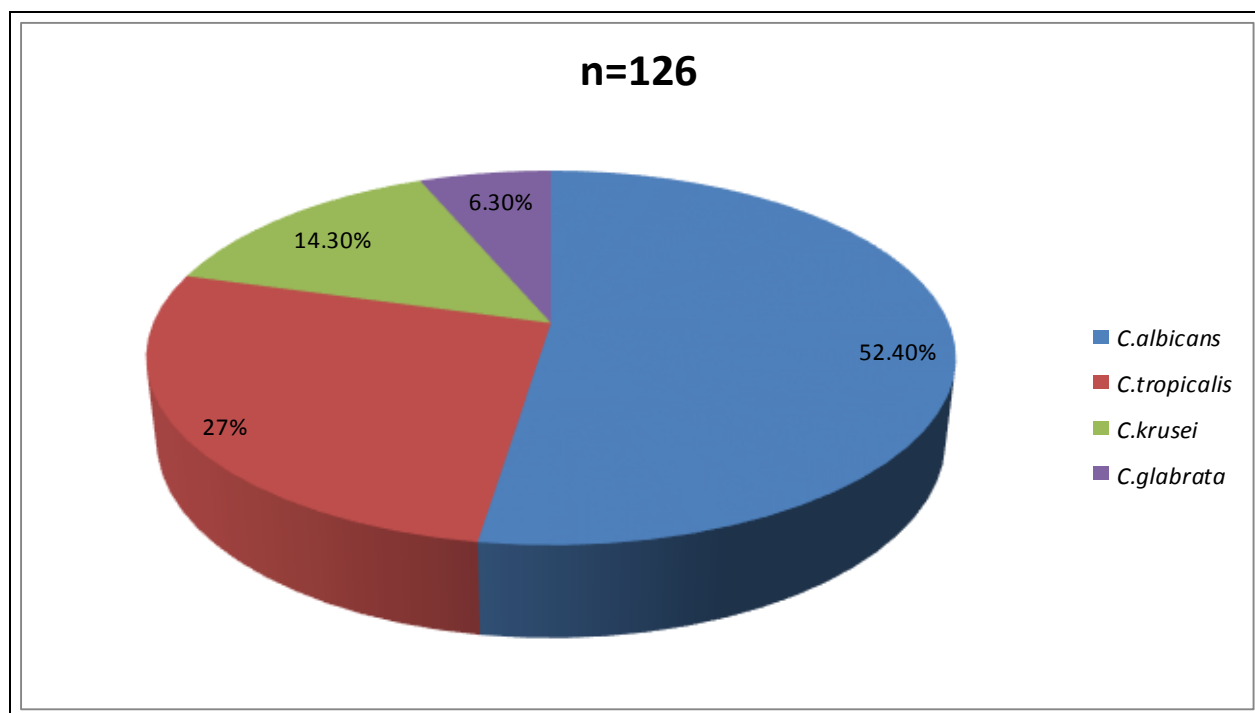
**Table.2** Antifungal sensitivity pattern of *Candida* isolates

| Antifungal agents | <i>Candida</i> species       |                                |                            |                              |
|-------------------|------------------------------|--------------------------------|----------------------------|------------------------------|
|                   | <i>C. albicans</i><br>(n=66) | <i>C. tropicalis</i><br>(n=34) | <i>C. krusei</i><br>(n=18) | <i>C. glabrata</i><br>(n=08) |
| Amphotericin B    | 100%                         | 100%                           | 96.7%                      | 98.1%                        |
| Nystatin          | 94%                          | 92.5%                          | 96.3%                      | 100%                         |
| Fluconazole       | 62.7%                        | 66%                            | 28.4%                      | R                            |
| ketoconazole      | 92.3%                        | 79%                            | 69.7%                      | 49.2%                        |

**Fig.1** Growth of *Candida* sp. in Sabouraud's Dextrose agar:



**Fig.2** Pie diagram showing distribution of different *Candida* species:



Our study showed that Amphotericin B was the most effective antifungal drug. This finding is similar with another study done by Maria Fatima Sugizaki *et al.*, (1998). The

next most effective antifungal drug was ketoconazole with 85.7% (108). Fluconazole was the least effective drug in our study with sensitivity of 38% (48). These findings

correlate with study done by Ananth kashid *et al.*, (2011).

In conclusion, *Candida* is one of the most commonly found nosocomial pathogen. The accurate species identification of *Candida* is important for treatment as Non-albicans *Candida* are resistant to most azole group of drugs and also because of rising anti-fungal resistance among different *Candida* species.

This study therefore emphasizes the need for rapid and precise identification of different *Candida* isolates to species level for effective treatment and management strategies. The present study also advocates the need for periodic surveillance of the antifungal drug susceptibility pattern of the prevalent various *Candida* species, as it would enlighten the judicious use of antifungal therapy in patients.

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