

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.504.094>

Speciation and Antifungal Susceptibility Pattern of *Candida* Isolated from Clinical Specimens

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ABSTRACT

Keywords

Candida,
CHROM agar,
Antifungal
susceptibility.

Article Info

Accepted:
24 March 2016
Available Online:
10 April 2016

Candidiasis is one of the major fungal infections among hospitalized and immunocompromised patients. Identification of *Candida* upto species level is required as there is increase in the incidence of non albican *Candida* infections. Increased incidence of antifungal resistance has also been reported. Hence, this study was aimed at speciation of *Candida* and their antifungal susceptibility from clinical specimens. A total of 93 *Candida* isolated from various clinical specimens. *Candida* Speciation was performed using CHROM agar and conventional methods. Antifungal susceptibility test was performed as per CLSI recommendations in document M44-A. *Candida albicans* (39.78%) was found to be the predominant species isolated from clinical specimens followed by non albican *Candida*. Among non albican *Candida*, *C.tropicalis* (22.58%) was predominant species isolated. *C.albicans* (45.95%) and *C.tropicalis*(38.09%) showed least sensitivity towards azole group. Majority of isolates remained susceptible to Amphotericin B. *Candida albicans* was found to be the predominant species isolated from clinical specimens. Among non albicans *Candida*, *Candida tropicalis* was the predominant species isolated. *Candida tropicalis* was least sensitive to azoles followed by *Candida albicans*.

Introduction

Candida species are the members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. Candidiasis is a common fungal disease found in humans affecting, mucosa, skin, nails and internal organs of the body. *Candida albicans* is by far the most common species causing infections in humans. (Fridkin, 2006) However, non albicans *Candida* species are also being implicated in recent years (Gullo, 2009).

Candida is the sixth most common isolated nosocomial pathogen, especially from urinary tract. It is the fourth most common cause of blood stream infection. (Fridkin, 1996) Predisposing factors for *Candida* infections are: prolonged use of antimicrobial agents, immunocompromised status, steroids, chemotherapy, and catheterization (Manchanda, 2011).

Species level identification of *Candida* is important for the treatment, as not all

species respond to the same treatment because of the problem of anti-fungal resistance. (Shivanand, 2011) Azole group of drugs, are commonly used in treating many forms of Candidal infections for a long time, however, their prolonged use has led to the development of drug resistance in *C.albicans* and other species. (Sachin, 2012) Amphotericin B, a polyene fungicidal agent, has been used for the treatment for invasive Candidal infections, but cost and dose related side effects limit its use. (Marchetti, 2003)

Hence, this study was aimed at speciation of candida and their antifungal susceptibility from clinical specimens.

Materials and Methods

A total of 93 Candida isolated from various clinical specimens submitted to the microbiology laboratory from different out patients and in patients departments of VM M C & H Karaikal, were included in the study. All suspected yeast colonies on sheep blood agar were confirmed by Gram staining. All such yeast isolates were sub cultured on chromogenic medium, HiMedia CHROM agar and incubated at 37° C for 24 hours and the species were identified by type and colour of the colonies on CHROM agar media as per manufacturer's instructions.

C.albicans – blue green

C.tropicalis – dark blue gray centre with pink halo

C. krusei – pink large rough spreading colonies with pale edge.

C.parapsilisis – pale cream coloured colonies.

C.dublinsiensis – dark green colonies.

All isolates were further inoculated on corn meal agar (CMA) by slide culture method to determine microscopic morphological features of various Candida species. (Marchetti, 2011)

Antifungal susceptibility test was performed as per CLSI recommendations in document M44-A. (Wayne Pa, 2004) Muller Hinton Agar supplemented with glucose and methylene blue, was prepared (Lee, 2001; Pfaller, 2004) Following antifungal discs were used amphotericin B (100 units), fluconazole (10mcg), clotrimazole (10mcg), itraconazole (10mcg), ketaconanole (10 mcg).

Quality control strain:

C.albicans (ATCC90028)

Statistical analysis was done by simple percentage method.

Results and Discussion

A total of 93 Candida spp. were isolated from various clinical specimens. Candida albicans (39.78%) was found to be the predominant species isolated from clinical specimens followed by non albican candida. Among non albican candida, *C.tropicalis* (22.58%) was predominant species isolated (Table.1) *C.albicans* (45.95%) and *C.tropicalis* (38.09%) showed least sensitivity towards azole group. Majority of isolates remained susceptible to Amphotericin B.(Table.2)

The pathogenesis of candida infections are extremely complex and probably varies with each species. Though over 100 species of candida have been recognized, only few have been found to cause human infections. (Krause, 2005) Candida albicans is generally considered as the major pathogen among the Candida species.

Table.1 Distribution of Candida Species among Various Clinical Specimens

Clinical specimen	<i>C.albicans</i>	<i>C.tropicalis</i>	<i>C.parapsilosis</i>	<i>C.krusei</i>	<i>C.dubiniensis</i>
urine	12(32.43%)	7(33.33)	4(23.53%)	4(30.77%)	-
Respiratory specimens	15(40.55%)	9(42.86%)	4(23.53%)	-	-
Pus/pus swabs	2(5.41%)	1(4.76%)	-	2(15.38%)	-
Vaginal swabs	5(13.51%)	3(14.29%)	1(5.88%)	1(7.70%)	1(20%)
CSOM	1(2.70%)	1(4.76%)	-	1(7.70%)	-
Blood	1(2.70%)	-	3(17.65%)	5(38.45%)	1(20%)
Stool	1(2.70%)	-	5(29.41%)	-	3(60%)
Total	37(100%)	21(100%)	17(100%)	13(100%)	5(100%)

Table.2 Antifungal Susceptibility Pattern of Candida Isolates

Candida species	Sensitive against Azole group	Sensitive against Amphotericin B
<i>C.albicans</i> (37)	17 (45.95%)	35(94.59%)
<i>C.tropicalis</i> (21)	8 (38.09%)	20(95.24%)
<i>C.parapsilosis</i> (17)	14(82.35%)	17(100%)
<i>C.krusei</i> (13)	11(84.61%)	13(100%)
<i>C.dubiniensis</i> (5)	3(60%)	5(100%)

An increase in the prevalence of non-albicans species has been noted during the last decades. The potential clinical importance of species-level identification has been recognized as Candida species differ in the expression of virulence factors and antifungal susceptibility. In the present study *C.albicans* (39.78%) was the predominant isolate. In our study the incidence of non albicans candida was 60.2%. Various studies stated that the incidence of non albicans candida ranges from 54-75%. (Golia, 2013) A study by Vijaya *et al.* (Vijaya, 2011) showed non-albicans candida (54.1%) to have a higher incidence than *C.albicans* (45.9%). A study by Manchanda *et al.* showed non- albicans candida (72.4%) to have a higher incidence than *C.albicans* (27.5%). Among the non albicans species, Candida tropicalis (37.5%)

was the predominant isolate followed by *C.parapsilosis* (30.3%). *C. tropicalis* is the most virulent of the non- albicans candida, this may be due to its ability to adhere to epithelial cells and its ability to secrete moderate levels of proteinases. (Moran, 2002)

Candida species were initially susceptible to ‘Azoles’, but now several species have developed resistance to the azoles. Widespread use of fluconazole for the prophylaxis and treatment of candidiasis has led to a reduction in the number of cases of infections caused by *Candida albicans* but has also resulted in the emergence of candidal infections caused by fluconazole-resistant *Candida non albicans*. (Gupta, 2001) In our study, candida tropicalis was least sensitive to azoles (38.09%) followed

by *Candida albicans* (48.95%). Patel *et al* have reported that Azole group showed 25.5% sensitive among *C.albicans* and 18.7% sensitive among *C. tropicalis* to fluconazole while in Amphotericin B, sensitivity varied from 75.6% to 100% to all isolated species of candida. (Patel, 2012) In our study, two strains of *Candida albicans* and one strain of *Candida tropicalis* showed resistance to amphotericin B. In another study by Deorukhkar *et al.* (Deorukhkar, 2013). It was seen that the resistant rate of *Candida* to fluconazole and amphotericin B was 27.3% and 5.8% respectively. The limitation in this study was that no other method was used to confirm the identity of the *Candida* isolates like the conventional carbohydrate fermentation and assimilation tests.

In conclusion, Non albicans *Candida* species are increasingly being isolated from clinical specimens. Among non albicans candida, *Candida tropicalis* was the predominant species isolated. *Candida tropicalis* was least sensitive to azoles followed by *Candida albicans*. Hence speciation and Susceptibility testing of the candida isolates plays an important role in the management of candidal infections. CHROM agar is a simple, rapid and inexpensive method for the identification of such candida species.

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How to cite this article:

Mohan, S., and Karthikeyan, D. 2016. Speciation and Antifungal Susceptibility Pattern of Candida Isolated from Clinical Specimens. *Int.J.Curr.Microbiol.App.Sci*.5(4): 820-824.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.504.094>