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### ANTIFUNGAL SUSCEPTIBILITY OF INTRAVENOUS CATHETER TIPS AND BLOOD DERIVED CANDIDA SPECIES BY DISC DIFFUSION METHOD

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**Abstract:** Background: Candidiasis is the most common fungal infection caused by various species of *Candida* with *Candida albicans* as representative species. The infection may be acute or chronic, superficial or deep, and its clinical spectrum is wide. The phenomenon of antifungal drug resistance is not restricted to pathogenic fungi, it also involves the commensal microbiota of humans. There is worldwide concern about the increase of drug resistant fungi. *Methods:* This prospective study was conducted in the Department of Microbiology at Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati from April 2012 to April 2013. A total of 50 intravenous catheter tips and blood samples were processed and the isolates were identified by using standard microbiological techniques and antifungal sensitivity was determined by Kirby Bauer disc diffusion method. Results: In the present study, three types of anti fungal drugs were used, which are amphotericin B, Fluconazole and Voriconazole. Among the collected 50 samples, 49(98%) were sensitive, 1(2%) sample is mild sensitive, whereas (0%) zero samples were resistant to Voriconazole. Whereas, 36(72%) were sensitive, 7(14%) were mild sensitive, 7(14%) were resistant to Fluconazole. Whereas, 40(80%) samples were sensitive and 10(20%) samples were resistant to Amphotericin-B. Most of the samples were sensitive to voriconazole than fluconazole and Amphotericin-B. Quality control limits for Anti fungal Agent is as per CLSI guidelines. Conclusion: Our study recommends further investigations on fungal resistance against various new generation triazoles using various susceptibility methods, rational use of antifungal and antibiotic drugs to prevent emerging resistance and development of new drugs with different mechanisms of action from those of the azoles.

**Keywords:** *Candida albicans*, Antifungal Susceptibility, Catheter tips



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## INTRODUCTION

Candida species are the most common cause of invasive fungal infections in humans, producing infections that range from non-life-threatening muco-cutaneous disorders to invasive disease that can involve any organ. Invasive candidiasis is largely a disease of medical progress, reflecting the tremendous advances in health care technology over the past several decades. The most frequently implicated risk factors include the use of broad-spectrum antibacterial agents, use of central venous catheters, receipt of parenteral nutrition, receipt of renal replacement therapy by patients in ICUs, neutropenia, use of implantable prosthetic devices, and receipt of immune suppressive agents (including gluco- corticosteroids, chemotherapeutic agents, and immune modulators.<sup>1</sup>

Candidiasis is the most common fungal infection caused by various species of Candida with *Candida albicans* as representative species. The infection may be acute or chronic, superficial or deep, and its clinical spectrum is wide. Candida is a yeast like fungus which reproduces by budding and exists as yeasts for part of their life cycle but buds fail to separate which results in the formation of pseudohyphae. In Candida species, during asexual reproduction, blastic ontogeny is seen, in which the conidium originates from narrow portion of the region, which swells before being cut off from parental cell by

septum.<sup>2</sup>The detection of Candida species in blood cultures is one of the most important advances in the recent diagnostic procedures. The LPCB mount is prepared from the isolated colonies to examine the presence of yeast cells and pseudohyphae.<sup>3</sup> In the present study we have to investigate antifungal resistance in the intravenous catheter tips and blood samples of SVIMS Hospital.

## MATERIALS AND METHODS:

The present study was carried out from April 2012 to April 2013 for a period of 12 months, during the study period total of 50 intravenous catheter tips and blood samples were collected and transported to the laboratory at the Institute within 3 hours after collection. Samples were inoculated onto MacConkey agar plate and incubated in ambient at 35°C for 18-24 hours before initial examination. The plates were examined for growth, The Candida isolates can be identified and speciated by standard protocols that include gram's staining and germ tube formation. Then, antifungal susceptibility testing was performed using Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye. The base medium can easily be supplemented either pre or post production, to contain the final concentration of 2% glucose and 0.5 µg/mL methylene blue dye. Although Mueller-Hinton agar is generally reliable for susceptibility testing, results obtained with

some brands and batches may, on occasion, vary significantly.<sup>(4)</sup> Antifungal susceptibility was determined by the Kirby Bauer's disk diffusion test in accordance with guidelines from Clinical Laboratory Standards Institute (CLSI) M-44A document.

**RESULTS:**

Total number of Candida isolates that were identified from various clinical specimens

was 50. Among these 36 isolates were from males and 14 isolates were from females. Out of these 50 Candida isolates 15 (22%) were *Candida albicans* species and 35(78%) were Candida nonalbicans species. In the present study, three types of anti fungal drugs were used, which are amphotericin B, Fluconazole and Voriconazole. Among the collected 50 samples, 36(72%) were sensitive, 7(14%) were mild sensitive, 7(14%) were resistant to Fluconazole.

**Total no.of samples collected(50)**

Candida albicans

15(22%)

Candida non albicans

35(78%)

Among the collected 50 samples, 49(98%) were sensitive, 1(2%) sample is mild sensitive, whereas (0%) zero samples were resistant to Voriconazole. Most of the samples were sensitive to voriconazole than fluconazole and Amphotericin-B. Among the collected 50 samples, 40(80%) samples were sensitive, where as 10(20%) samples were resistant to Amphotericin-B. Quality control limits for Anti fungal Agent is as per CLSI guidelines.

Quality control limits for the *Candida albicans* for Amphotericin-B is 10-15 mm. The average of all Candida non albicans species was taken for the determination of Quality control limits of Candida non albicans species and it was taken as 12 mm. In this study, we observed that anti-fungal resistance is increasing in the candida species.

**RESISTANCE PATTERN OF TOTAL ISOLATES (50)**

ANTI FUNGAL DRUG	SENSITIVE	MILD SENSITIVE	RESISTANT
AMPHOTERICIN-B	40(80%)	0	10(20%)
FLUCONAZOLE	36(72%)	7(14%)	7(14%)
VORICONAZOLE	49(98%)	1(2%)	0

**DISCUSSION:**

There is worldwide concern about the emergence of antifungal resistance of intravenous catheter tips and blood derived candida species. It may represent an enormous and constant reservoir of resistant genes, potentially transferable to virulent candida species. The present study was under taken to find out the prevalence of antifungal resistance in candida species. A total number of 50 samples were included in the study. In this study it was noted that resistance patterns are more for the Amphotericin-B. Out of these 50 Candida isolates 15 (22%) were *Candida albicans* species and 35(78%)were Candida non albicans species.

Yet, Wingard in a review of 1591 cases of Candida infection published in 37 reports between the year 1952-1992, noticed that non-Candida albicans species were the causative agents in 46% of systemic infections. *Candida tropicalis* accounted for 25% of infections. Vang et al evaluated 230 blood isolates and 344 non blood isolates of Candida species at National Taiwan University hospital Taipei. They reported that the proportion of *C. tropicalis* increased from 14% during 1981-1993 to 23% during 1996-2002. <sup>(5)</sup> P faller et al. studied fluconazole susceptibility of *C. glabrata* and considered a high agreement between agar-based and MD methods, with 64.7 and 52.3%, respectively for D Dand ET<sup>(6)</sup>. In our present study, we observed an

increasing resistance pattern for amphotericin-b and fluconazole.

**CONCLUSION:**

It can be concluded that candida species causes Oral thrush, Esophagitis, Cutaneous candidiasis, Vaginitis, Balanitis, Systemic candidiasis. It necessitates prevention of candida infections by conducting the infection control programmes and implementation of surveillance for this emerging antifungal resistance phenomenon. To control or reduce the rate of carriage for these organisms, effective measures should be taken to prohibit the sale of antifungals without medical consultation. Every hospital should monitor the antibiogram profile of organisms time to time to serve as a basis for empirical therapy in emergency situations. Our study therefore recommends the following: Further investigations on fungal resistance against various new generation triazoles using various susceptibility methods. Rational use of antifungal and antibiotic drugs to prevent emerging resistance. Development of new drugs with different mechanisms of action from those of the azoles. Expansion of the spectrum of activities of the current antifungal drugs to cover a wide spectrum of Candida infections.

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