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# Invitro susceptibilities of Candida isolates to Fluconazole and Voriconazole determined by disc diffusion in a tertiary care centre, South India

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## Abstract:

The in vitro susceptibilities of fluconazole and voriconazole against a total of 95 Candida isolates during the one year study period August 2010 – July 2011 in a tertiary care centre , South India was determined by the agar disc diffusion test using the Clinical and Laboratory Standards Institute (CLSI) M44-A2 guidelines. Out of 95 total isolates, we got 43 *Candida tropicalis*, 31 *Candida albicans* and 21 *Candida parapsilosis*. 3.2 % of total isolates were fluconazole resistant but all were sensitive to voriconazole. None of them show Susceptible- dose- dependence (S-DD). 100% of *C.albicans* were sensitive to both fluconazole and voriconazole. 2.3% of *C.tropicalis* isolates and 9.5% of *C.parapsilosis* were resistant to fluconazole. The study demonstrated a better in vitro activity of voriconazole, against the fluconazole resistant strains of Candida. But as the fluconazole resistance is rare and the species which are intrinsically resistant to fluconazole like *C.krusei* and *C.glabrata* were not prevalent , flucoconazole can still be used as the drug of choice for empirical therapy of uncomplicated candidiasis. Voriconazole can be reserved for salvage treatment of refractory candidiasis caused by resistant strains.

**Key words:** Candidiasis, Fluconazole, Voriconazole

## Introduction

Candida species represent the most common cause of fungal infections [1]. *C.albicans* remained the predominant agent of candidiasis including candidemia till recently and is usually susceptible to azoles such as fluconazole [1-3]. For the past two decades, infections with Non- *albicans* Candida species (NAC) which are comparatively less fluconazole susceptible has markedly increased [4,5]. Some species like *C. glabrata* and *C.krusei* are intrinsically resistant to the drug [6-8]. Fluconazole is an azole group of antifungal agent which got approved in 1990s and has been widely used for both treatment and prophylaxis of fungal infections, due to its clinical efficacy, safety, bioavailability in both

oral and parenteral formulations as well as due to its cost effectiveness [9,10]. Due to its limited spectrum of antifungal activity and resistance noticed in immunocompromised hosts, second generation triazoles have been developed [9]. The first of these new agents to receive approval from US Food and Drug Administration (FDA) was voriconazole [8]. It broadens the available therapeutic options for the treatment of invasive candidiasis due to its potential activity against some fluconazole – resistant Candida species and strains [8,9]. Although voriconazole can bind to the target enzyme with high affinity, it has more side effects, drug interactions and cost compared to fluconazole, inspite of its expanded spectrum [10].

A knowledge of the epidemiology of fluconazole resistant species and strains of Candida and their corresponding voriconazole susceptibilities may be helpful in formulating an institutional azole based empirical therapy for uncomplicated candidiasis.

## Material and Methods

A descriptive study was conducted over a period of twelve months in the department of Microbiology, at a tertiary care centre. A total of 95 consecutive pure cultures of Candida from various clinical samples submitted to the hospital microbiology laboratory from different clinical departments were studied. The isolates were speciated by conventional biochemical methods like sugar fermentation and assimilation, corn meal agar morphology and colour on Hichrome agar [11-13]. The fluconazole and voriconazole susceptibility patterns were studied as per CLSI disc diffusion guidelines M44-A2 using fluconazole disc 25µg (SD232, Himedia, Mumbai) and voriconazole disc 1µg (SD277, Himedia, Mumbai) [14].

Inoculum was prepared by picking 5 distinct colonies of approximately 1mm from 24 hours old culture on Sabouraud's dextrose agar. Colonies were suspended in 5ml of sterile saline and adjusted the turbidity to 0.5 McFarland standard [14].

The medium used was Muller Hinton Agar (M173,Himedia, Mumbai) + 2%glucose and 0.5 µg/ml Methylene blue dye medium which was poured in about 4mm depth. Discs were applied with centres 24mm apart and incubated at $37^0$C. Plates were examined after 24 -48 hours [14]. The sensitivity patterns were determined according to CLSI guidelines. **(Table: 1 and Figure :1)**

## Results:

Out of 95 Candida isolates there were 43 *C. tropicalis*, 31 *C. albicans* and 21 *C.parapsilosis*. Three (3.2%) of the total isolates were resistant to fluconazole but were 100% sensitive to voriconazole. None of them showed Susceptible- dose- dependence (S-DD). All of the 31 *C. albicans* isolates were sensitive to both fluconazole and voriconazole. Out of 43 *C. tropicalis* isolates, one (2.3%) was resistant to fluconazole. Among the 21 *C.parapsilosis* isotates, two (9.5%) were fluconazole resistant.

## Discussion

The study aimed to determine the invitro susceptibilities of Candida isolates to fluconazole and voriconazole and to compare both. Even though NAC species were predominating, fluconazole resistance was rare in our clinical isolates of Candida. *C.krusei* and *C.glabrata* which were intrinsically resistant to fluconazole were not isolated during our study period. Voriconazole seemed to be superior to fluconazole with a better susceptibility in the fluconazole resistant strains also. This may be due to the more effective binding of voriconazole to cytochrome P450 isoenzyme of Candida species [15]. The result we got for the three species of Candida confirmed most of the previously published data from various Indian studies conducted in different parts like Bangalore, Mangalore and New Delhi [16-18]. All these studies showed a predominance of NAC species and apart from a mild difference in the susceptibility rates, all showed a higher susceptibility to voriconazole than fluconazole [16-18]. But it is interesting to notice that the resistance to voriconazole is more common for *C.tropicalis* and *C. parapsilosis* strains than to *C. albicans* [16-19]

Results from the latest ARTEMIS DISK Global Antifungal Surveillance Study of Candida species shows susceptibility rates of 90.2% for fluconazole and 95% for voriconazole [20]. Fluconazole sensitivity to *C.albicans*, *C.tropicalis* and *C. parapsilosis* were 98.0%, 91.0% and 93.2% respectively. Voriconazole was more active than fluconazole against most species of Candida with the exception of *C. tropicalis* (91.0% S to fluconazole versus 89.5% S to voriconazole [20]. The predominant species and their resistance patterns showed geographical trends, like a higher resistance to fluconazole and voriconazole by *C. albicans* in North America and higher reisistance by *C. tropicalis* in the Asia-Pacific region compared to other areas

[20-22]. *C. parapsilosis* appears to show a slight trend toward increasing resistance over time [20-22]. An increasing fluconazole resistance was also seen with some emerging pathogens like *C. guilliermondii*, *C. lusitaniae*, *C. sake* and *C. pelliculosa*, *C. rugosa* and *C. norvegensis* [20-22].

It is comforting to know that both of these triazoles remain active against many of our isolates and voriconazole remains sensitive to the fluconazole resistant strains also. Intrinsic fluconazole resistant species are not endemic. Fluconazole can be continued as the first line antifungal agent for treating suspected cases of uncomplicated candidiasis. Voriconazole can be reserved for refractory cases of candidiasis. But whenever there is lapses in infection control precautions coupled with broad use of fluconazole, more fluconazole-resistant strain of endemic species and other rare species may emerge, complicating the situation.

## Conclusion and Suggestions

The changing epidemiology of candidiasis highlights the need for continued surveillance of species distribution and susceptibility both locally and on a regional and international basis, in order to optimise therapy and outcome. Each institution should develop guidelines for empiric therapy based on the epidemiology of their own hospital environment.

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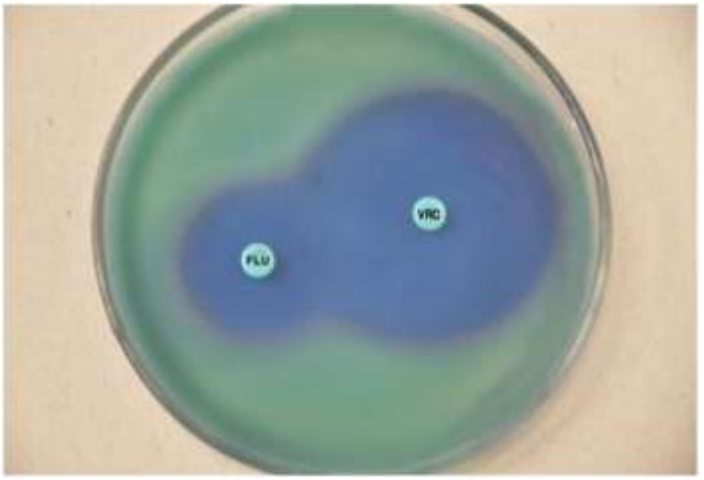
**Table 1: Interpretative guideline as per CLSI** [14]

| Antifungal agent | Disc content (µg) | Resistant (mm) | Susceptible-dose-dependent (mm) | Susceptible (mm) |
|---|---|---|---|---|
| Fluconazole | 25 | ≤ 14 | 15-18 | ≥ 19 |
| Voriconazole | 1 | ≤ 13 | 14-16 | ≥ 17 |

**Figure 1: Antifungal susceptibility test**




**Fluconazole (FLU) >19mm (S), Voriconazole (VRC) > 17mm (S)**

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