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Comparative Study of MIC Values of Fluconazole, Voriconazole and Flucytosine to *Candida* species

Saroj Golia, Vivek Hittinahalli*, Mallika Reddy K. and Sujatha Karjigi

Dr. B.R. Ambedkar Medical College, K.G. Halli, Bangalore - 560 045, India.

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Candida albicans **and** ***Candida non albicans*** **are emerging important causative agents of fungal infections. The study was carried out to identify the various species of** ***Candida*** **using Chrom agar and cornmeal agar and compare their susceptibility pattern to fluconazole, voriconazole and flucytosine by microbroth dilution methods. Positive samples for candidiasis where collected from 122 patients at Dr BR AMC from may 2011 to July 2012, processed by routine methods, chrom agar media and corn meal agar was used to speciate and antifungal susceptibility done by microbroth dilution method.** ***C.albicans***[44]**,** ***C.tropicalis***[32]**,** ***C.parapsilosis***[24]**,** ***C. glabrata***[16] **and** ***C. krusei***[6] **were isolated. MIC values of fluconazole,voriconazole and flucytosine to** ***C.albicans***, ***C.tropicalis,*** ***C.parapsilosis***, ***C.glabrata***, ***C.krusei*** **were determined. There is increasing incidence of** ***C.non albicans*** **species infections. There also has been increase in the MIC values of fluconazole for non albicans candida., MIC values of voriconozole were not found to be increased and were in the range of 0.007-0.125ug/ml. Hence remains the drug of choice among all the azoles. MIC values of flucytosine were in the range of 0.002-0.75ug/ml except for** ***C. krusei*** **where it is >32ug/ml. This suggests that flucytosine also remains a good drug for treatment of candidiasis however this drug is more toxic.**

Key words: *Candida,*chromagar, Cornmeal agar, Flucanazole,Voriconazole, Flucytosine.

The increasingly frequent use of cytotoxic, antibacterial and immunosuppressive drugs required to treat both malignant and non-malignant diseases has recently been associated with a rise in the incidence of serious fungal infections[1]. Serious fungal infections carry considerable morbidity and mortality[2-3]. Since molecular techniques are too expensive, use of Chrom agar for species differentiation would be of benefit for easy and rapid speciation[6,8].The study is therefore to determine the *Candida non albican* species and their MIC values to fluconazole, voriconazole and flucytosine, in our setup.

* To whom all correspondence should be addressed.

MATERIALS AND METHODS

It is a prospective study conducted at Dr B R AMC from 2011 May to 2012 July. The inclusion criteria were from patients having symptoms of candidiasis where other causes were ruled out.

Speciation

Samples first obtained were Gram stained, inoculated on to the SDA agar slopes (Sabouraud dextrose agar) incubated at 37°C for 24 hours. Germ tube test was done and further were classified as *Candida albicans* and non albicans. The germ tube positive isolates were further incubated at 45°C to look for the growth.They were also inoculated on to Chrom agar(Hi media) from the SDA slopes and incubation at 37°C. Identification was made by colour and morphology of the colonies as per the manufacturer's instructions. Simultaneously the *Candida* strains were inoculated on to corn meal agar (CMA) and slide cultures were put up for

chlamydospore, blastoconidia formation. Flow chart represents the method followed for identification. Table 1 represents different colours produced by various species on Chromogenic agar, and also the pattern of chlamydospore and blastoconidia formation.

For MIC Suseptibility Testing

Inoculum were prepared from 1-3 days old cultures on SDA. Suspensions were adjusted by using 0.5 McFarland standard. Antifungal susceptibility testing was carried out by microbroth dilution method as per CLSI guidelines M27-A2. Stock solutions were prepared, serial 2-fold dilutions were made in RPMI 1640 medium(buffered to pH 7.0 with 0.165 M MOPS) of fluconazole, Voriconazole and flucytosine .The powders were obtained from Hi media[9-10].

RESULTS

A total of 122 patients positive samples of *Candida* species were collected ,the goal of the study was to show an increase in the incidence of *Candida non albicans* infections. Fig. 1 represents the different colours obtained of different species and Fig. 2 represents different pattern and arrangement of blastoconidia andchlamydospore formation. Table 2 shows the number and percentage of species of *candida* obtained.*C.albicans*(36%) was the most common species among all the isolates. Other *Candida* species isolated were,*C. tropicalis*(26.2%) , *C. parapsilosis*(19.6%) , *Candida glabrata* (13.1%), and *C. krusei* (4.9%) Thus the overall incidence of non-albicans *Candida* species was 63.9% (78/122). Table3 represents MIC distribution of fluconazole, voriconazole and flucytosine . *Candida albicans* showed the lowest MIC values and *C. krusei* showed highest MIC values among all the antifungals. After antifungal testing the results were obtained as shown in Table 3.

For fluconazole MIC values < 8 ug/ml were considered to be susceptible ,whereas isolates with MIC > 64 ug/ml were considered to be resistant. Isolates with MICs between 16-32 ug/ml were fluconazole susceptible-dose dependant (S-DD)[9,10]. For voriconazole MIC values ≤1 µg/ml were considered to be susceptible, where as isolates with MIC ≥ 4 µg/ml were resistant[13,15]. For flucytosine criterion of ≥ 32 µg/ml were considered to be resistant using the CLSI 85 M27-A3 24h.[13-14,16].

Table 1. *Candida* speciation on Chromogenic agar and arrangement

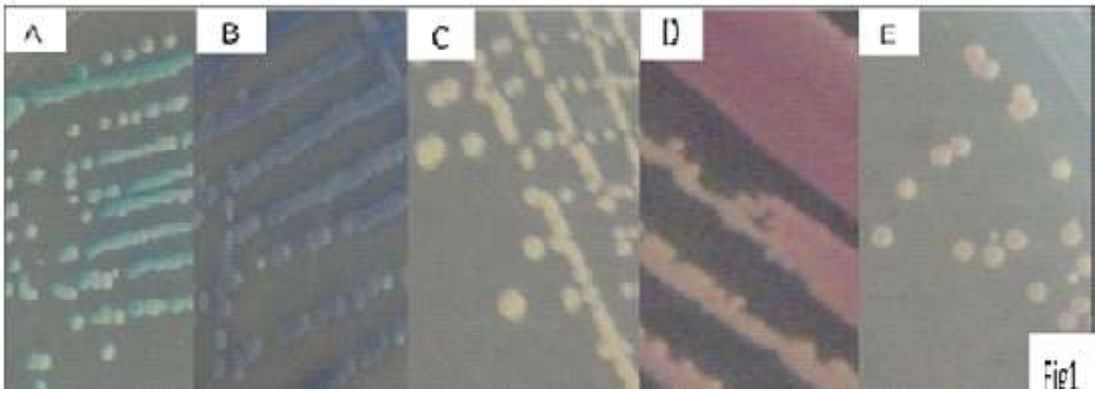
	Name of isolates	Colour on Chrom agar	Type and arrangement of blastoconidia &chlamydospore formation
A	*C. albicans*	Light green	large, thick-walled chlamydospore, usually terminal and present singly or in small clusters along with clusters of round blastoconidia.
B	*C. tropicalis*	Dark blue	oval blastoconidia singly or in small groups all along , long pesudohyphae.
C	*C. parapsilosis*	Pale cream colour	short, pencil-like pseudohyphae with blastoconidia arranged singly along pseudohyphae
D	*C. glabrata*	pink	pseudohyphae with blastoconidia forming cross-matchstick appearance.
E	*C. krusei*	Pale pink with white edge rough and spreading	yeast cells only

Table 2. Percentage of *Candida* species

Name	Number	Percentage
C.albicans	44	36%
C.tropicalis	32	26%
C.parapsilosis	24	20%
C.glabrata	16	13%
C.krusei	06	5%
TOTAL	122	100

Table 3. Shows the MIC range of fluconazole,voriconazole&flucytosine

Name	Flucanazole MIC Range (ug/ml)	Voricanazole MIC Rangerange(ug/ml)	Flucytocine MIC Range(ug/ml)
C. albicans	0.125-0.5	0.007–0.125	0.004–0.125
C. tropicalis	0.5-2	0.007–0.125	0.002–0.75
C. parapsilosis	0.5-4	0.007-0.125	0.003-0.19
C. glabrata	16-32	0.007–1	0.006-4
C.krusei	32-64	0.007–2	>32



A-*C.albicans*,B-*C.tropicalis*,C-*C.parapsilosis*,D-*Cglabrata*& E-*C.krusei*

Fig. 1. *Candida* speciation on Chromogenic agar

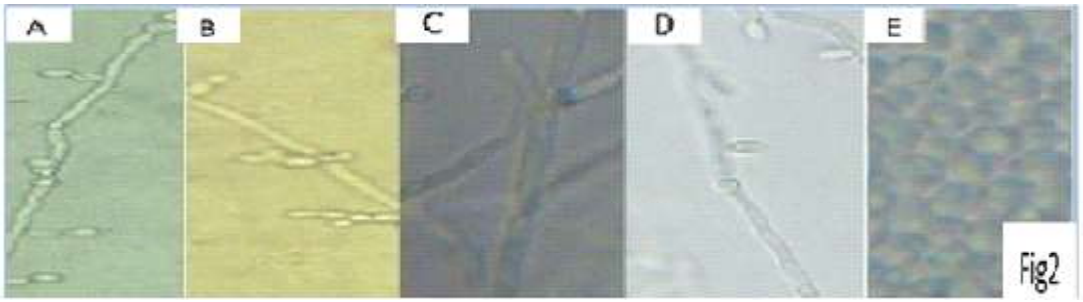


Fig. 2. Type and arrangement of blastoconidia &chlamydospore formation

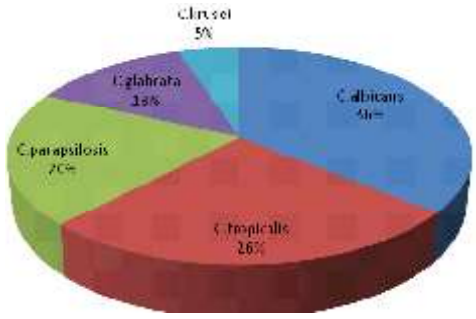
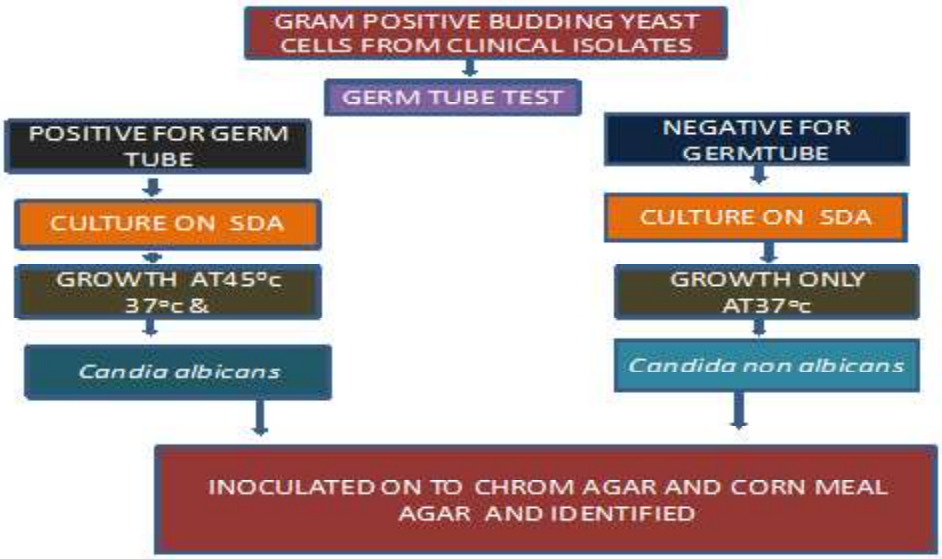


Fig. 3 After antifungal testing the following results were obtained as shown in table 3



Flow Chart 1.

DISCUSSION

The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility [6],[8]Infections with these yeast species also have a direct impact on the choice of empiric antifungal therapy and clinical outcome. Chromogenic medium was able to identify *Candida albicans, C. tropicalis, C. krusei, C. parapsilosis*. Chromogenic medium was also helpful in isolating more than one species in single patient[5-7,10]. Antifungal drug of choice is first based on *Candida* species. But MIC values testing will play an increasingly important role while selecting antifungal drug dosage. Standardized methods for MIC testing have been available for many years. The Clinical and Laboratory Standards Institute (CLSI) standardized broth micro dilution method remains a reference for antifungal susceptibility and MIC testing .MIC values of voriconozole were not found to be increased more than 0.007-0.125ug/ml hence remains the drug of choice among azoles.MIC values of flucytosine were in the range 0.002-0.75ug/ml except for *C krusei*>32ug/ml. This suggests that flucytosine also remains a good drug for treatment of systemic candidiasis.

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