Al Ameen J Med Sci 2013; 6(2):163-166 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

Speciation of *Candida* using chromogenic and cornmeal agar with determination of fluconazole sensitivity

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Abstract: *Objective and background:* Candidiasis is an important cause of fungal infections. This study was carried out to determine the species incidence using chrom agar & cornmeal agar, susceptibility pattern to fluconazole by microbroth dilution methods of yeasts isolated from the clinical samples. *Method:* Positive samples for candidiasis where collected from 112 patients at Dr B R AMC from April 2010 to April 2011, processed by routine methods, chrom agar media and corn meal agar was used to speciate and antifungal susceptibility done by microbroth dilution method. *Results; C.albicans*(40), *C.tropicalis*(30), *C.parapsilosis* (22), *C.krusei* (5), *C glabrata*(15) were obtained. Susceptibility to flucanazole showed MIC *C.albicans* (0.125-0.5ug/ml), *C.tropicalis* (0.5-2ug/ml*), C.parapsilosis* (0.5-4 ug/ml), *C.glabrata* (16-32ug/ml) *C.krusei* (32-64ug/ml), *Conclusion:* Increasing incidence of non albicans species infection. The MIC of non albicans also showed increased values.

Keywords: *Candida*, Chrom agar, cornmeal agar, fluconazole

Introduction

Infections due to *Candida* species are increasing as the number of immunocompromised patients are increasing, undue usage of antibiotics, and cancer patients are more susceptible to fungal infections. Identification of *Candida* to species level is definitely warranted as there is increase in the incidence of *Candida non albicans* infections [1]. The antifungal susceptibility of fluconazole has changed against the *Candida* species, *Candida non albicans* are being more resistant to flucanazole [2]. Since molecular techniques are too expensive using of Chrom agar for species differentiation would be of benefit for easy and rapid speciation [3-4]. The study helps to determine the non albican species and their increased MIC levels to fluconazole in the setup.

Material and Methods

It is a prospective study conducted at Dr B R AMC from 2010 April to 2011 April. The inclusion criteria were from patients having symptoms of candidasis where other causes were ruled out. Samples first obtained where Gram stained, inoculated on to the SDA agar slopes (Sabouraud dextrose agar) incubated at 37^0c for 24 hrs. Germ tube test was done and further were classified as albicans and non albicans. The germ tube positive was further incubated at 45^0c to look for the growth. They were also inoculated on to Chrom agar(hi media) from the SDA slopes, identification was made by colour and morphology of the colonies as per the manufacturer's instructions table 1 represents the colours produced by various species fig 1 shows the type and colour of the colonies on Chromagar after incubation at 37^0c.Simultaneously the *Candida* strains were inoculated on to corn meal agar (CMA) for chlamydospore, blastoconidia formation and arrangement slide cultures fig 2 shows morphology on CMA, table 2 represents kind and pattern of chlamydospore & blastoconidia formation. Flow chart 1 represents the method followed for identification

Table-1: *Candida* species colour on Chromogenic media

	Name	Colour on chrom agar
A	*C albicans*	Light green
B	*C tropicalis*	Dark blue
C	*C parapsilosis*	Pale cream colour
D	*C glabrata*	pink
E	*C krusei*	Pale pink with white edge rough and spreading

Table-2: Type and arrangement of blastoconidia &chlamydospore formation		
	Name	**Type and arrangement of blastoconidia &chlamydospore formation**
A	*C albicans*	large, thick-walled chlamydospore, usually terminal and present singly or in small clusters along with clusters of round blastoconidia.
B	*C tropicalis*	oval blastoconidia singly or in small groups all along , long pesudohyphae.
C	*C parapsilosis*	short, pencil-like pseudohyphae with blastoconidia arranged singly along pseudohyphae
D	*Ckrusei*	yeast cells only
E	*C glabrata*	pseudohyphae with blastoconidia forming cross-matchstick appearance.

Fig-1: *Candida* speciation on Chromogenic agar

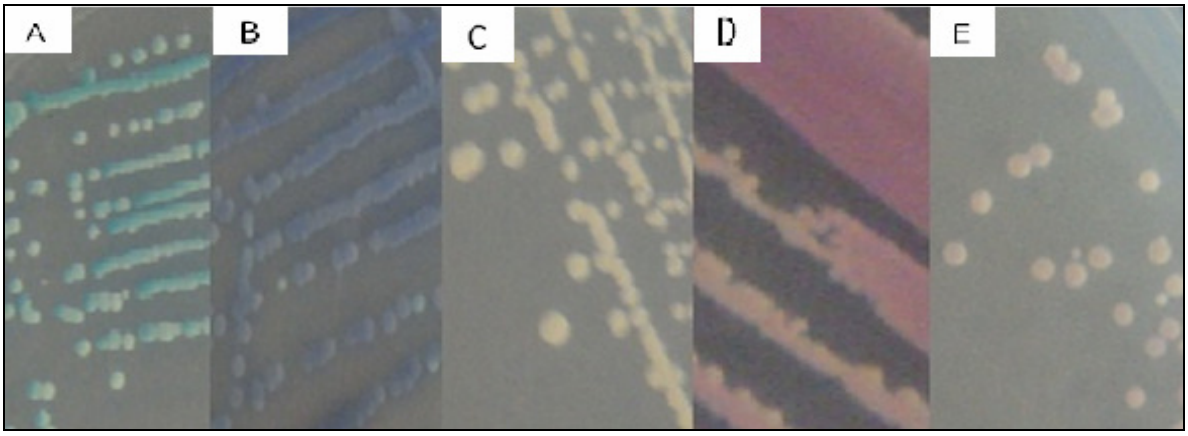
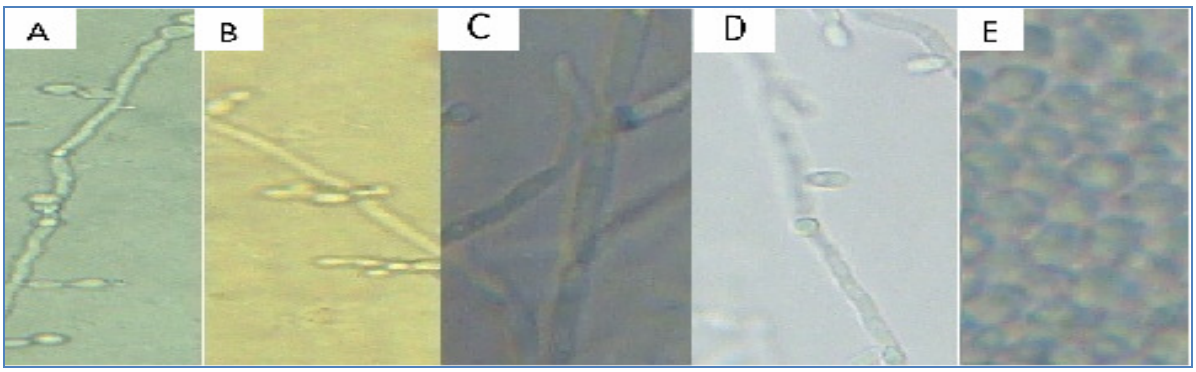


Fig-2: Type and arrangement of blastoconidia &chlamydospore formation



Inocula 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. Serial dilution of fluconazole was done (0.125,0.25,0.5,1,2,4,8,16, 32,64)ug/ml.[5] Sterile microdilution plates (96-u-shaped wells) were used. Rows 1-10 contained the series of drug dilutions in 100 ul volumes starting with the concentration of 0.125 ug/ml. 100 ul of inoculum suspension were added to each well. The eleventh well was the control well, 100 ul of inoculum suspension and 100 ul of drug free medium were added. The plates were covered, incubated at room temperature and examined after 48, 72 hours incubation [5-6] Break points isolates of MIC Isolates with MIC < 8 ug/ml were considered to be susceptible to fluconazole, 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) [6-7].

Results

A total 112 patient positive of *Candida* species were collected the goal of the study was to show there is increase in the incidence of *Candida non albicans*. Table 3 shows the number and species of candida (chart 2represents table 3) *C. albicans* (35.7%) was the most common species among the isolates. Other *Candida* species isolated were, *C. tropicalis*(26.7%), *C. parapsilosis*(19.6%), *Candida glabrata(13.6%)* ,and *C. krusei (4.4%*) Thus, the overall prevalence of non-albicans candida species was per cent (72/112)64.2%. There was increase in MIC levels against the levels of fluconazole, *Candida albicans* showed the lowest MIC levels while *C krusei* showed highest MIC values table 4 shows MIC of all species and MIC 90 was also done. Chart 3 represents table 4.

Chart-2: Candida species distribution

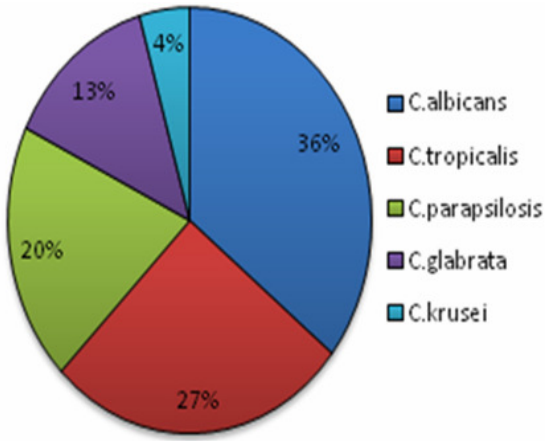
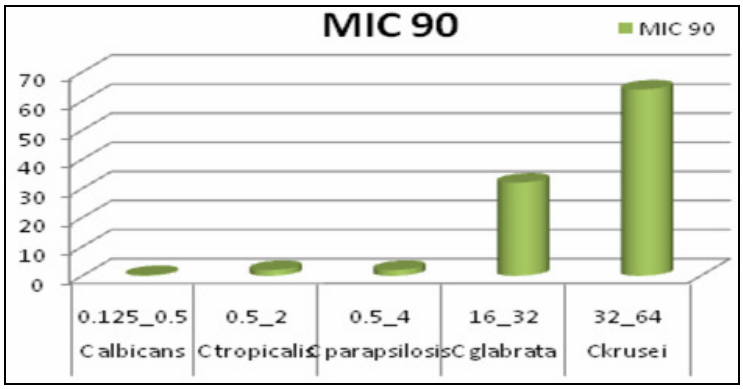


Table-3: Distribution of *Candida* species		
Name	**Number**	**Percentage**
C.albicans	40	36%
C.tropicalis	30	27%
C.parapsilosis	22	20%
C.glabrata	15	13%
C.krusei	5	4%
Total	112	100

Table-4: MIC distribution to flucanazole		
Name	**MIC Range (ug/ml)**	**MIC 90 (ug/ml)**
C. albicans	0.125-0.5	0.25
C. tropicalis	0.5-2	2
C. parapsilosis	0.5-4	2
C. glabrata	16-32	32
C.krusei	32-64	64

Chart-3: MIC distribution of *Candida* species to flucanazole



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 [3-4]. Infections with these yeast species also have a direct impact on the choice of empiric antifungal therapy and clinical outcome. The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the antifungal susceptibility [3-4]. Chromogenic medium was able to identify *Candida albicans, C. tropicalis, C. krusei, C. parapsilosis.* Chromogenic medium was also helpful in identifying "multi-species" yeast infections [3, 8-10]. Antifungal choice is first based on *Candida* species identification, but antifungal susceptibility testing will play an increasingly important role when selecting antifungal drug dosage to use. Standardized methods for antifungal susceptibility testing have been available for many years. The Clinical and Laboratory Standards Institute (CLSI) standardized broth microdilution method remains a reference for antifungal susceptibility testing. Indeed, clinically relevant interpretative breakpoints are available and quality control strains are validated. Nevertheless for the CLSI method, serial twofold dilutions ranging from 0.125 to 64 ug/ml for fluconazole [6-7,10]thus the present study shows using Chromagar & cornmeal agar helps us to speciate with ease, shows that increasing incidence of *Candida non albicans*. MIC levels also helps to determine the dosage pattern of each species.

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