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Antifungal Susceptibility of Candiduria Isolates by E Test Method

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A B S T R A C T

Candida species other than *Candida albicans* have now emerged as an important cause of UTI and some of which exhibit reduced susceptibility to commonly used antifungal agents. It causes urinary tract infections in healthy individuals, but common in hospital settings. The aim of this study was to investigate species distribution and antifungal susceptibility profile of *Candida* species causing candiduria. The present study determined the Antifungal susceptibilities of the *Candida* isolates from urine against Fluconazole and Itraconazole and Ketoconazole by E-test according to the CLSI reference method M27-S4. Glucose methylene Mueller- Hinton agar (GM-MH) was used as the test medium. Out of 84 isolates of *C. albicans* 23(27%) showed maximum resistance to fluconazole followed by ketoconazole. 18(21%). Fluconazole resistance was more in *C. tropicalis* (11) followed by *C.albicans* (9). Resistance to ketoconazole was common in *C. tropicalis* (7). Itraconazole resistance was also higher in *C. tropicalis* (7). Therefore, it can be concluded that non *Candida albicans* have emerged as an important cause of urinary tract infections. Susceptibility results shows that there is emerging fungal resistance to azoles particularly fluconazole and new generation triazoles.

Introduction

Candida species are emerging as an important cause of hospital-acquired infections. The wide spectrum of diseases ranges from superficial, mucocutaneous infections to invasive diseases involving internal organs as well as bloodstream infections Nayman *et al*., (2011). Technological advances in medicine have created new opportunities for *Candida* to gain access to the circulation and deep tissues. The shift of *Candida* species from commensal to pathogen is facilitated by a number of virulence factors such as adherence to host tissues and medical devices, biofilm formation, and secretion of extracellular hydrolytic enzymes Kauffman *et al*., (2000).The incidence of urinary Candidiasis continues to rise in proportion to a growing number of patients with innately azole-resistant *non- Candida albicans* species. The prolonged use of broad spectrum antibiotic therapy, urinary catheterization, diabetes mellitus, invasive devices and prolonged hospital stays are risk factors associated with candiduria (Kauffman *et al*., 2011).

Effective treatment requires both early diagnosis and prompt initiation of therapy against fungal infection. Antifungal susceptibility testing represents a means of predicting therapeutic concentration of antifungal drugs used to treat a variety of candida infection. Importantly, many non-albicans species have decreased susceptibility to antifungal agents over the past decades. Increasing resistance to azoles and amphotericin B has also been reported by Wadha *et al*., (2015).

E-test for determination of sensitivity of *Candida* species to antifungal agent as it is accurate and simple. E-test is a stable agar gradient strips that consist of drug concentration scale. It determines the MIC of different antifungal agents in a rapid and simple method.(CLSI M27-S4) The purpose of this study was to determine the in vitro susceptibility of Candiduria isolates by E-test. All the results will documented with WHONET software.

Materials and Methods

The study was conducted in the department of Microbiology, Travancore Medical College, Kollam from September 2014 to December 2015 during which 84 cases of candiduria isolates were collected. The *Candida* isolates were sub cultured on Sabouraoud's Dextrose Agar and Hi-CHROM candida agar medium and identified by Germ tube test, sugar fermentation and assimilation tests (Fisher *et al*., 2011).

Processing of urine samples

An inoculating wire loop calibrated to hold 0.01ml of urine was sterilized in a Bunsen flame. The loop was allowed to cool. The loop was inserted vertically into the urine sediment in the test tube and was inoculated on Blood agar, Sabouraud dextrose agar. Quantitative culture with candida colony counts of $>10^4$ cfu/ml in patients without indwelling catheters and $>10^3$ cfu/ml of urine in patients with indwelling catheters (Passos *et al.*, 2005).

Antifungal Drug Susceptibility Testing

E-TEST Minimum Inhibitory Concentration (MIC)

It gives a quantitative measurement of the MIC of clinical isolates of *Candida* species. The E-strips **(**AB Biodisk) consisted of drug concentrations ranging from 0.016 µg/ml to 256 µg/ml. Antifungals used are fluconazole, ketoconazole and itraconazole.

Isolated colonies were suspended in sterile normal saline and turbidity adjusted to 0.5 McFarland standard. Using sterile cotton swab the suspension was inoculated on to RPMI agar (supplemented with 2% glucose and buffered with MOPS, 0.165M, and pH 7.0) plates (150 mm diameter) and allowed to dry for 10 to 15 minutes before applying the E-test strips. The plates were incubated at 35ºC and minimum inhibitory concentration (MIC) was recorded after 48 hrs. The interpretive susceptibility breakpoints as recommended by Clinical Laboratory Standards Institute (CLSI M27-S4) were used. The determination of the MICs of antifungal agents is based on reading the lowest concentration at which the border of the elliptical inhibition zone intercepted the scale on the strip. Azoles being fungistatic, so appearance of colonies throughout the discernible inhibition ellipse is ignored.

Results and Discussion

A total of 84 isolates of *Candida* species were included in the study. *C.albicans* were

(39%), *C.tropicalis* (29%), *C.krusei* (19%), *and C.glabrata* (13%). Table 1 Table 2 shows the antifungal resistance pattern of *Candida* isolates. Out of 84 isolates of *C. albicans* 23(27%) showed maximum resistance to fluconazole followed by ketoconazole 18 (21%). Fluconazole resistance was more in *C. tropicalis* (11) followed by *C.albicans* (9). Resistance to ketoconazole was common in *C. tropicalis* (7).Itraconazole resistance was also higher in *C. tropicalis* (7). *C. krusei and C. glabrata* were inherently resistant to fluconazole.

Table.1 *Candida* spp isolated from Urine sample

Species	No of isolates (%)
C.albicans	**33(39%)**
C.tropicalis	**21(25%)**
C.krusei	**14(16%)**
C.glabrata	**11(13%)**
C.parapsilosis	**5(6%)**
Total	**84**

Table.2 Antifungal Resistance

Candida species	Fluconazole	Ketoconazole	Itraconazole
***C.albicans*(33)**	**9**	**3**	**4**
***C.tropicalis* (21)**	**11**	**7**	**5**
***C.krusei*(14)**	*****	**2**	**3**
***C.glabrata*(11)**	*****	**5**	**1**
***C.parapsilosis*(5)**	**3**	**1**	**0**
Total	**23(27%)**	**18(21%)**	**14(16%)**

***** Inherently resistant

The incidence of candiduria caused by non-albicans *Candida* is emerged in the last few years Manisha *et al*., (2011). Our study also supported the same trend. The advancement in medical techniques has contributed this increase in candiduria. Among this catheterization procedure is the one which increases the chances of urinary tract infection. The indiscriminate use of antibiotics and antifungals also lead to the increased candidial infections Yashavanth *et al*., (2013)**.** In our study isolation rate of non albicans *Candida* was 51(61%) which is higher than *C. albicans* (39%), this finding correlates with the study of Iman *et al*., (2010).

In our study fluconazole resistance by E-test method was higher 23(27 %) than other antifungals used. Fluconazole resistance in candiduria is an alarming concern because fluconazole is a drug having high concentration in urine. The long term use of azoles also associated with the increased resistance. Susceptibility of *C.albicans* to fluconazole was (27%) with MIC < 16µg/ml, which is similar to the reports of other studies of Abhijit (2012), Ooga *et al*., (2011). *Non albicans candida* also showed increased resistance to fluconazole. Susceptibility results shows that there is emerging fungal resistance to azoles particularly fluconazole and new generation

triazoles particularly itraconazole. Rahul Kumar *et al*., (2016).

In conclusion, in our study, non *Candida albicans* were the predominant candidurial isolates. Therefore, it can be concluded that non *Candida albicans* have emerged as an important cause of urinary tract infections. Its isolation from clinical specimens can no longer be ignored as non pathogenic isolate nor can it be dismissed as a contaminant. Proper surveillance of these fungal pathogens is important to improve quality of care in tertiary care setting. More studies should be carried out as the commonly isolated *Candida* spp and their antifungal susceptibility patterns in hospital settings.

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