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STUDY OF ANTIFUNGAL DRUGS SUSCEPTIBILITY OF CANDIDA ISOLATES

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ABSTRACT

Fungal infections are a growing medical problem requiring prompt diagnosis with species identification and early adapted antifungal therapy. Among the increasing fungal infections, candida species are the most common in the recent few decades. Fluconazole, a triazole derivative has become the drug of choice for the treatment of candidiasis in immunocompromised patients. Antifungal susceptibility of candida isolates to fluconazole and voriconazole was performed by disc diffusion method. As many as 14.28% candida isolates showed resistance to fluconazole. Some of the non-albicans candida species like C. glabrata (40%) showed more resistance to fluconazole. Candida isolates showed 2.39% resistance to voriconazole.

Keywords :- Candida, Antifungal therapy, Fluconazole, Voriconazole.

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INTRODUCTION

Fungal infections are a growing medical problem requiring prompt diagnosis with species identification and early adapted antifungal therapy.[1-4] Among the increasing fungal infections, candida species are the most common in the recent few decades.[5-9] Candida species colonize the mucosal surfaces of all humans soon after birth and the risk of endogenous infection is ever-present.[10,11] But the organism become pathogenic only when the normal bacterial flora is disturbed by antibiotics or other factors that produce fungal overgrowth.[12]

The strains of Candida albicans can be differentiated at the phenotypic level by physiological tests and by detection of phenotypically expressed macromolecular structures. For this various combined tests have been suggested. Of these combined methods, resistotyping and morphotyping are the most suitable methods which are easily available.[13]

Resistotyping was first developed for strain delineation, pathogenesis and epidemiological studies,[14] reported by McCreight and Warnock with modifications. This method is convenient and easy for biotyping of large number of C. albicans isolates.[15] Morphotyping has also been studied as an epidemiological tool. This is a method of evaluating fringe and surface characteristics of streak colonies, shown to have good discriminatory capacity. This method is able to relate strains of proven virulence with distinct morphotypes.[16,17]

Fluconazole, a triazole derivative has become the drug of choice for the treatment of candidiasis in immunocompromised patients. The rise in incidence of

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resistance to antifungal drugs is more alarming. Although amphotericin B and flucytosine continue to be effective, the loss of susceptibility to azoles, is a matter of great concern.[13]

With introduction of azoles, the cause of candida infection shifted from Candida albicans to non albicans species, and developed resistance to fluconazole. Candida glabrata and Candida krusei have been observed to be 4 to 32 fold less susceptible than C. albicans to fluconazole.[18] Hence identification of candida isolates upto species level from various clinical specimens and their antifungal susceptibility testing is necessary.

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology. A total of 210 candida isolates of suspected cases of candidiasis from various clinical specimens were included in the study.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed by antifungal disc diffusion susceptibility testing of Yeasts as per the CLSI guidelines.[19,20] Standard strains of candida species - Candida albicans - ATCC 90029, Candida krusei - ATCC 6258, Candida parapsilosis - ATCC 22019. Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue dye medium was used. The pH of the medium kept in between 7.2 to 7.4 Discs Used are Fluconazole (Himedia) 25µg and Voriconazole (Himedia) 1µg. Colonies were suspended in sterile distilled water. Inoculum is standardized to 0.5 McFarland using a densitometer.

Sterile swab soaked in inoculum and streaked all over the plate through an angle of 600C. Finally, swab was passed over the edge of the agar surface is to be made on the culture plates. Discs were applied. Plates incubated at 35oC for 24 hours.

Zones of inhibition were measured, interpreted and recorded as-

Susceptible (S), zone diameters of

- ≥19 mm (fluconazole)
- ≥ 17 mm (voriconazole)

Resistant (R) - ≥ 14 mm (fluconazole)

- ≥ 13 mm (voriconazole).

RESULT AND DISCUSSION

Out of 210 Candida strains, maximum isolates i.e. 125 (59.52%) were of C. albicans. Among the non albicans candida, most of the isolates were of C. tropicalis 50 (23.84%) followed by C. glabrata 13 (6.19%). Other nonalbicans species like C. krusei 8 (3.80%), C. parapsilosis 6 (2.85%), C. guilliermondii 5 (2.38%), C. kefyr 3 (1.42%) were also isolated from clinical Specimens (Table 1).

Table 1. Distribution of candida species (n=210)

Candida species	No. of isolates (%)
C. albicans	125 (59.52)
C. tropicalis	50 (23.84)
C. glabrata	13 (06.19)
C. krusei	08 (03.80)
C. parapsilosis	06 (02.85)
C. guilliermondii	05 (02.38)
C. kefyr	03 (01.42)
Total	**210 (100.0)**

Table 2. Age wise distribution of candida species (n=210)

Age in years	*Candida albicans*	Candida tropicalis	Candida glabrata	Candida krusei	Candida parapsilosis	Candida guillier mondii	Candida kefyr	Total
0 – 28days	10	05	04	02	02	-	-	23 (10.95)
29days-12month	01	-	-	01	-	-	-	02(0.95)
1-10	07	06	01	01	-	-	-	15 (07.14)
11-20	09	06	-	-	-	02	01	18 (08.57)
21-30	24	08	01	01	-	01	-	35 (16.66)
31-40	38	13	03	01	02	-	01	58 (27.61)
41-50	30	06	02	01	01	02	-	42 (20.03)
>50	06	06	02	01	01	-	01	17 (08.09)
Total	125	50	13	08	06	05	03	210

Table 3. Distribution of candida species in different specimen

Specimen	Candida species							
	Candida albicans	**Candida tropicalis**	**Candida glabrata**	**Candida krusei**	**Candida parapsilosis**	**Candida guillier mondii**	**Candida kefyr**	**TOTAL (%)**
Urine	61	13	06	02	03	-	01	86 (40.95)
Oral swab	27	17	02	-	-	-	01	47 (22.38)
Vaginal swab	22	10	02	03	02	02	01	42 (20.00)
Blood	07	04	02	03	-	02	-	18(8.57)
sputum	04	02	01	-	-	-	-	07(3.35)
Pus	01	03	-	-	01	-	-	05(2.38)
Peritoneal fluid	01	02	-	-	-	01	-	03 (01.42)
Synovial fluid	02	-	-	-	-	-	-	02 (00.95)
TOTAL	**125(59.52)**	**50(23.84)**	**13(6.19)**	**08 (3.80)**	**06(2.85)**	**05(2.38)**	**03 (1.42)**	**210 (100)**

Table 4. Morphotypes of Candida albicans (n=125)

Morphotyping Code	C. albicans isolates (%)
000 0	51 (40.8)
222 1	10 (08.00)
732 5	09 (07.20)
323 4	08 (06.40)
000 6	07 (05.60)
722 0	07 (05.60)
322 0	05 (04.00)
732 5	05 (04.00)
224 0	04 (03.20)
734 0	04 (03.20)
324 4	03 (02.40)
522 5	03 (02.40)
523 8	03 (02.40)
551 0	03 (2.40)
524 2	02 (01.60)
721 0	01 (0.80)
TOTAL	**125**

Table 5. Resistotypes of Candida albicans (n=125)

Resistotypes	C. albicans isolates (%)
-B - - F-	57 (46)
-B - - - -	25 (20)
A B - - - -	14 (11)
- - - - F-	12 (10)
A - - - F -	09 (07)
A – C - - -	05 (04)
A – C – F - G	03 (02)
TOTAL	**125 (100)**

Table 6. Antifungal susceptibility of candida isolates

Candida Species	No. of Isolates Resistant to	
	Fluconazole (%)	**Voriconazole (%)**
C. albicans (125)	15 (12.0)	03 (2.4)

C. tropicalis (50)	05 (10.0)	00
C. glabrate (13)	02 (40.0)	01 (20.0)
C. crusei (08)	08 (100.0)	01 (12.5)
C. parapsilosis (06)	00	00
C. guilliermondil (05)	00	00
C. kefyr (03)	00	00
Total (210)	**30 (14.28)**	**05 (2.39)**

Table 2 shows that in all the age groups, C. albicans was the most common species contributing to 125 (59.52%) followed by C. tropicalis 50 (23.84%) and C. glabrata 13 (6.19%). Out of total 13 (6.19%) isolates of C. glabrata, maximum isolates were seen in the age group of neonates.

Of the 86 (40.95%) urinary isolates, 61 (70.93%) were of C. albicans followed by 13 (15.11%) were C. tropicalis and 6 (6.97%) were C. glabrata. Out of 47 (22.38%) isolates of oral swabs, 27 (57.44%) were of C. albicans followed by 17 (36.17%) were of C. tropicalis. From vaginal swabs, 42 (20.00%) candida strains were isolated, of these 22 (52.38%) were C. albicans, 10 (23.80%) were C. tropicalis and 3 (7.14%) were C. krusei. Blood specimens showed, 7 (38.88%) C. albicans, 4 (22.22%) C. tropicalis and 3 (16.66%) C. krusei.

Table 4 shows that '000 0' was the most common morphotype of C. albicans contributing to 51 (40.8%) isolates, followed by '222 1', '732 5' in 10 (8.00%) and 09 (07.20%) isolates respectively. '721 0' was the least common morphotype seen. Discontinuous fringes like 222 1, 224 0, 322 0, 323 4, 324 4 were isolated from blood, sterile body fluids and other invasive infection. Sixteen different morphotypes were identified. Most common morphotype was '000 0' contributing to 51 (40.8%), followed by '222 1' (8.00%) and '732 5' (07.20%). In our study special morphological markers, like discontinuous fringes, have been observed from blood, other sterile body fluids.

Resistotyping of C. albicans

Among the 125 isolates of C. albicans, - B - - F - was the most common resistotype observed in 57(46%) isolates, followed by ' - B - - - -' resistotype in 25 (20%) and 'A B - - - - ' resistotype in 14 (11%) isolates. In this study out of 125 isolates of C. albicans, 77% isolates were resistant to boric acid and 65% were to malachite green, 24% to sodium selenite, 6% to cetrimide, 2% to copper sulphate. All the isolates were sensitive to sodium periodate.

Antifungal susceptibility of candida isolates

Table 6 shows that out of total 210 candida isolates, 30 (14.28%) isolates were resistant to fluconazole. Out of 125 isolates of C. albicans, 15(12%) isolates showed resistance to fluconazole. Out of 50 isolates of C. tropicalis and 13 isolates of C. glabrata, 5(10%) of C. tropicalis and 02(40%) of C. glabrata were resistance to fluconazole respectively. 100% resistance to fluconazole was observed in C. krusei. C. guilliermondii, C. parapsilosis and C. kefyr showed 100% sensitivity to fluconazole. Out of total 210 candida isolates, 5 (2.39%) isolates were resistant to voriconazole. Out of 125 C. albicans, 03(2.4%) showed resistance tovoriconazole. Out of 8 isolates of C. krusei and 13 isolates of C. glabrata only one isolate of each showed resistance to voriconazole. C. tropicalis, C. guilliermondii and C. kefyr showed 100% sensitivity to voriconazole.

Antifungal susceptibility testing was performed by antifungal disc diffusion susceptibility testing of yeasts as per the CLSI guidelines.93,94 In our study out of total 210 isolates, (Table 14) 14.28% isolates were resistant to fluconazole. 12% of C. albicans, 10% of C. tropicalis were resistant to fluconazole. C. glabrata showed 40% resistance to fluconazole. 100% resistance was observed in C. krusei. All the isolates of C. krusei were resistant to fluconazole which is in agreement with the general observation that this species has innate resistance to the azoles group of antifungals.

CONCLUSION

Total 210 candida strains were isolated from different clinical specimens of suspected cases of candidiasis. There were 57.64% males and 42.38% females. Male preponderance was seen. Morphotyping suggested by Hunter et al21 was applied to C. albicans isolates. Sixteen different morphotypes of C. albicans were identified. '000 0' was the common morphotype contributing to 40.8%. Resistotyping of C. albicans was done according to Medcraft's modification of McCreight and Warnock's method.24 Seven different resistotypes were reported. - B - - F - was the commonest resistotypes, followed by - B - - - - resistotype. Antifungal susceptibility of candida isolates to fluconazole and voriconazole was performed by disc diffusion method. As many as 14.28% candida isolates showed resistance to fluconazole. Some of the non-albicans candida species like C. glabrata (40%) showed more resistance to fluconazole. Candida isolates showed 2.39% resistance to voriconazole. For characterization of C. albicans, resistotyping and morphotyping methods can be used. These methods are simple and easy to perform in the laboratory.

Resistance to commonly used antifungal agents is a cause of concern. Our study noted high fluconazole resistance in candida isolates. Hence, it is now a high time

that peripheral microbiology laboratories should perform antifungal susceptibility testing for candida isolates routinely. It is a simple disc diffusion method and can be performed in resource constrained laboratories also. This will keep vigilance on drug resistance in candida, a commonest fungal cause of human infection. This will also help in control of the resistance in a long way.

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