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Full Length Research Paper

Isolation, characterisation and antifungal susceptibility pattern of *Candida albicans* and non *albicans Candida* from Integrated counseling and testing centre (ICTC) patients

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The present study was designed to isolate, characterize and perform the antifungal susceptibility pattern for *Candida albicans* and non *albicans Candida* among the patients who visited the Integrated Counseling and Testing Centre (ICTC) at Sendamangalam Primary Health Centre, Namakkal. Vaginal swabs were obtained from 125 women in the age group of 16 to 49 years and a detailed clinical history was obtained with a short questionnaire. A standard procedure was employed for the isolation and identification of *Candida* spp. of the 125 samples collected, *Candida albicans* was isolated in 18 (14.4%) cases and *non albicans Candida* was isolated in 10 (8%) cases which include *Candida tropicalis* (3.2%), *Candida glabarata* (2.4%), *Candida dubliniensis* (1.6%) and *Candida krusei* (0.8%). The positivity was assessed over the various socio-demographic profile, risk factors and symptoms. Among the occupational group, daily wagers were significantly associated ($p < 0.00$) with the *C. albicans* positivity and the symptom dysuria was statistically significant ($p < 0.03$). The socio-demographic variables had no significant association with non *albicans Candida*. Antifungal susceptibility test revealed that resistance to Amphotericin B is infrequent, and all the isolates showed resistance to Clotrimazole and Itraconazole. Most of these isolates showed resistance to more than three antifungal agents and to assess their resistance pattern, ten different patterns were obtained. Therefore, this work emphasizes the need for testing the antifungal susceptibility tests for the *Candida* isolates to control the spread of new resistant strains in future.

Key words: *Candida albicans*, non *albicans Candida*, integrated counseling and testing centre (ICTC) patients, antifungal agents, resistance.

INTRODUCTION

Reproductive tract infections (RTIs) are the major public health problems among women especially in developing countries. The World Health Organization (WHO) estimates that approximately 340 million new cases of curable sexually transmitted infections (STIs) occur every year and majority of them in developing countries (Bala and Sood, 2010). Apart from the sexually transmitted diseases (STD), non-sexually transmitted genital infections like Candidiasis can have serious detrimental effect to the women health. The prevalence data have a key role in control strategies for RTIs and STIs. In India, routine surveillance of these infections is not carried out and estimation of the total incidence is quite difficult. Even in the modern advances in medicine, there is a rise in the incidence of fungal infections especially those due to *Candida species*. *Candida albicans* is the most

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common fungal pathogen that cause superficial and deep seated Candidiasis (Gullo, 2009). *Candida* may be either a commensal or a pathogen of the vagina and they colonize the mucosal surfaces of all humans soon after birth and the risk of endogenous infection is always present. The changes in the vaginal microenvironment are generally necessary for *Candida* to induce pathological changes associated with clinical symptoms. Depending on the age, locality and socio-economic status, the frequency of vaginal yeast isolates has been reported 5 to 48.4% in healthy women (Gross et al., 2007). Carriage rate of *Candida* spp. tends to increase with age. The prevalence of *Candida* spp. in prostitutes was 20.6% (Gross et al., 2007). Vaginal candidiasis is the second most common cause of vaginitis after anaerobic bacterial vaginosis (Kikani et al., 2010). Data of incidence of vaginal candidiasis suggest approximately two-thirds of women experience at least one episode during their lifetime and nearly 50% of women have multiple episodes. Vulvovaginal candidiasis are mainly caused by *C. albicans* (Richter et al., 2005), but nowadays the incidence with other species of *Candida* called non-*albicans Candida species* also reported. *Candida* infections were treated effectively with azole-based antifungal drugs and it can be complicated by the emergence of drug resistant yeasts (Casalinvovo et al., 2004). The emergence of these drug resistant strains, both chromosomal and plasmid borne, reinforce the need for the study of these pathogens and the surveillance of its susceptibility to antibiotics commonly used for therapy (Palomares et al., 1990). Close monitoring of the antimicrobial susceptibility and its resistance mechanism is essential in an environment of rapidly changing resistance patterns. Therefore, the present investigation was undertaken to isolate *C. albicans* from ICTC patients, characterization and antifungal susceptibility patterns of *Candida* isolates.

MATERIALS AND METHODS

Patients

Vaginal samples were collected from the ICTC patients of Primary Health Centre, Sendamangalam, Namakkal district. The study included patients with or without clinical symptoms of vulvovaginitis (pruritis, dysuria or vulval burning). A pre-designed, structured questionnaire sheet was filled in sampling site, and study subjects were explained with symptoms and risk factors of disease. All study subjects who informed and written consent where included in the study.

Sampling procedure

From each patient, two swabs were used to collect the specimen from the vagina or cervix avoiding the contamination of other organisms. One was used for direct smear examination and other was inoculated on Sabouraud's dextrose agar and subjected to incubation at 37°C aerobically. Direct smear examination was also performed with 10% KOH mount and Gram staining procedure. Identification tests were carried out by standard laboratory procedures.

Identification

The growth of *C. albicans* and other non *albicans Candida* on SDA was confirmed by Gram staining and Gram positive budding yeast-like cells were further confirmed with germ tube test in human serum. The obtained isolates were characterized by inoculation into SDA broth, Hichrome Candida agar and corn meal agar. Other biochemical tests like sugar fermentation, sugar assimilation, urease test and incubation at 45°C were also performed for the confirmation and differentiation of the *Candida* species.

Antifungal susceptibility testing

Antifungal susceptibility testing was performed by NCCLS M44-A disc diffusion method (Wayne, 2004). Inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24 h old culture of *Candida* isolates. Colonies were suspended in 5 ml of sterile saline and its turbidity was adjusted visually with the transmittance to that produced by a 0.5 McFarland standard. Inoculation of test plates were done with a sterile cotton swab dipped into the suspension. The dried surface of a sterile Mueller-Hinton agar plate was inoculated by evenly swabbed over the entire agar surface. Antifungal discs (Itraconazole, Clotrimazole, Fluconazole, Ketoconazole, Amphotericin B and Nystatin) were dispensed onto the surface of the inoculated agar plate. The plates were inverted and placed in an incubator at 35°C. The plates were examined after 20 to 24 h. The zones of inhibition were measured and the results were recorded.

RESULTS

A total of 28 yeast-like isolates were obtained from 125 vaginal specimens collected from the ICTC patients. The isolates showed Gram positive oval yeast-like cells in Gram staining and growth was obtained on SDA. The isolates includes *C. albicans* (n = 18), *Candida tropicalis* (n = 4), *Candida glabrata* (n = 3), *Candida dubliniensis* (n = 2) and *Candida krusei* (n = 1) (Table 1). Thus, the overall prevalence of *Candida albicans* was 14.4% and non *albicans Candida* was 8%. The species were identified and differentiated by the standard procedures (Table 2). The age of patients at their ICTC Clinic visit ranged between 16 to 49 years. The prevalence was found to be more in the age group 16 to 35 years (Table 3) for *Candida* species. Tables 4 and 5 showed the association between socio-demographic profile of the study population and prevalence of vulvovaginal candidiasis. It was found that majority of *Candida* infections occurred in illiterates and married women. The positivity in both *C. albicans* and non *albicans Candida* were found to be more in females worked as daily wagers when compared to housewives and others. Occupation as daily wagers was significantly associated with *C. albicans* positivity at 0.006% level. Majority of the positive cases were belonged to a lower socioeconomic status with poor life-style. None of the risk factors were statistically significant.

Table 1. Incidence of *Candida* spp.

S/N	*Candida* species	Number of Isolates	Percentage (%)
1	*Candida albicans*	18	14.4
2	*Candida tropicalis*	04	3.2
3	*Candida glabrata*	03	2.4
4	*Candida dubliniensis*	02	1.6
5	*Candida krusei*	01	0.8

Table 2. Characterization of *Candida* spp.

S/N	Species	Colour on chrome agar	Chlamydospores on corn meal agar	Pellicle on SDA broth	Germ tube test	Growth at 45℃
1	*C. albicans*	Light green	+	-	+	+
2	*C. tropicalis*	Dark Blue green	-	Small pellicle	-	-
3	*C. glabrata*	Dark pink	-	-	-	-
4	*C. dubliniensis*	Pale color	++	NA	++	-
5	*C. krusei*	Pink centre with white edge	-	Thick pellicle	-	-

Table 3. Prevalence of *Candida* spp. over the age distribution.

Age group	No of subjects	Prevalence		Total
		C. albicans	Non *C. albicans*	
16-25	34	02	01	03
26-35	70	11	05	16
36-45	15	04	02	06
> 46	06	01	02	03
Total	125	18	10	28

Surprisingly, most of the women who had the *C. albicans* infection knew about the usage of condoms (77.7%), but it was less in non *albicans Candida* species (40%). The use of contraceptives was significantly associated with *C. albicans*. 55.5% of the positive *C. albicans* subjects frequently use the antifungal and antibacterial agents but it was less (30%) in non *albicans Candida*. Following an explanation of the symptoms, 60% of the positive women had experienced the symptoms like smell in vaginal discharge and curd like vaginal discharge, nearly 40% had vaginal lesions or sores in genitalia, and nearly 50% had burning micturition, pain in lower abdomen, itching and dysuria or vulval burning. Among the symptoms, dysuria showed the statistical significance. Other variables were not positively associated. Table 6 showed the susceptibility test results for the 18 isolates of *Candida albicans*, revealed that resistance to Amphotericin B is infrequent. Resistance to Clotrimazole and Itraconazole occurred in 100% of the isolates (Table 7). The non *albicans Candida* showed 100% resistance to Nystatin, Itraconazole and Clotrimazole (Table 8). Amphotericin B had the effect over 90% of the isolates and Ketoconazole is effective for all the *non albicans Candida*.

DISCUSSION

Despite therapeutic advances, vulvovaginal Candidiasis remains a common problem worldwide, affecting all stratas of society (Akpan et al., 2011). Candidial infections are more dynamic than other diseases prevailing in the community. Their epidemiological profile varies from country to country and from one region to another within a country depending upon ethnographic, demographic, Socio-economic and health factors. The clinical pattern was also a result of the interaction among pathogens, the behaviours that transmit them and the effectiveness of preventive and control interventions (Thappa and Kaimal, 2010).

With multiple antifungals and varying susceptibility patterns of *Candida*, it has now become necessary to perform antifungal susceptibility testing real-time and make reports available to the clinician for effective therapeutic outcome (Srinivasan and Kenneth, 2005). The present study was carried out to isolate and characterize the *Candida* spp. from ICTC patients. Among 125 women studied, the prevalence of *Candida* species were 22.4%. The similar pattern of isolation was

Table 4. Prevalence of *Candida albicans* over the different variables of study subjects.

Variables	Prevalence of *Candida albicans*				Total		Chi-square test	P value	Odds ratio	95% confidence interval	
	Positive		Negative								
	No	%	No	%	No	%				Lower limit	Upper limit
Educational status											
Literate	7	13.7	44	86.3	51	100	0.031	0.85	0.91	0.33	2.53
Illiterate	11	14.9	63	85.1	74	100					
Marital status											
Married	15	17.4	71	82.6	86	100	2.069	0.15	2.54	0.69	9.33
Unmarried	3	7.7	36	92.3	39	100					
Occupation											
Daily wages	13	27.1	35	72.9	48	100	10.208	0.006***	-	-	-
Housewife	4	6.2	61	93.8	65	100					
Others	1	8.3	11	91.7	12	100					
Socio economic status											
Low	13	14.6	76	85.4	89	100					
Moderate	5	13.9	31	86.1	36	100	0.010	0.91	1.06	0.35	3.23
High	-	-	-	-	-	-					
Knowledge about STDs											
Yes	5	10.4	43	89.6	48	100	1.003	0.31	0.57	0.19	1.72
No	13	16.9	64	83.1	77	100					
Known the usage of condoms											
Yes	14	26.4	39	73.6	53	100	10.776	0.001***	6.10	1.88	19.84
No	4	5.6	68	94.4	72	100					
Partner working											
Local	11	15.9	58	84.1	69	100					
Mofffusal	4	23.5	13	76.5	17	100	0.545	0.46	0.62	0.17	2.25
Foreign	-	-	-	-	-	-					
Hospitalization											
Yes	1	4.2	23	95.8	24	100	2.523	0.11	0.21	0.03	1.70
No	17	16.8	84	83.2	101	100					

Table 4. Contd.

Extra marital sex											
Yes	1	5.6	17	94.4	18	100	2.233	0.13	0.23	0.03	1.85
No	14	20.6	54	79.4	68	100					
Pre-marital sex											
Yes	3	23.1	10	76.9	13	100	0.886	0.34	1.94	0.48	7.87
No	15	13.4	97	86.6	112	100					
Any contraceptive methods											
Copper-T	2	11.1	16	88.9	18	100	20.623	0.000***	-	-	-
Pills	1	3	32	97	33	100					
Condoms	8	57.1	6	42.9	14	100					
Others	4	19	17	81	21	100					
Use of anti-fungal/antibacterial agents											
Yes	10	21.7	36	78.3	46	100	3.180	0.07	2.47	0.90	6.79
No	8	10.1	71	89.9	79	100					
Smell/curd like vaginal discharge											
Yes	11	20.3	43	79.7	54	100	2.749	0.09	2.34	0.84	6.51
No	7	9.9	64	90.1	71	100					
Vaginal lesions /sores in genitalia											
Yes	7	20.6	27	79.4	34	100	1.450	0.22	1.89	0.66	5.35
No	11	12.1	80	87.9	91	100					
Burning micturination											
Yes	10	16.7	50	83.3	60	100	0.480	0.48	1.43	0.52	3.89
No	8	12.3	57	87.7	65	100					
Pain in lower abdomen											
Yes	10	18.9	43	81.1	53	100	1.490	0.22	1.86	0.68	5.09
No	8	11.1	64	88.9	72	100					
Itching											
Yes	10	20.8	38	79.2	48	100	2.616	0.10	2.27	0.83	6.23
No	8	10.4	69	89.6	77	100					

Table 4. Contd.

Dysuria/vulval burning											
Yes	12	21.8	43	78.2	55	100	4.3846	0.03**	2.98	1.04	8.54
No	6	8.6	64	91.4	70	100					

***, Significant at 5% level; **, Significant at 1% level.

Table 5. Prevalence of non *albicans Candida* over the different variables of study subjects.

Variable	Prevalence of Non *albicans Candida*				Total No	Chi-square test	P value	Odds ratio	95% confidence interval	
	Positive		Negative							
	No	%	No	%					Lower limit	Upper limit
Educational status										
Literate	3	5.9	48	94.1	51	0.52	0.47	0.60	0.15	2.43
Illiterate	7	9.5	67	90.5	74					
Marital status										
Married	9	10.5	77	89.5	86	2.28	0.13	4.44	0.54	36.35
Unmarried	1	2.6	38	97.4	39					
Occupation										
Daily wages	9	18.8	39	81.2	48					
Housewife	1	1.5	64	98.5	65	12.27	-	-	-	-
Others	0	0	12	100	12					
Socio economic status										
Low	8	9	81	91	89					
Moderate	2	5.6	34	94.4	36	0.41	0.52	1.68	0.34	8.32
High	-	-	-	-	-					
Knowledge about STDs										
Yes	3	6.3	45	93.7	48	0.32	0.57	0.67	0.16	2.71
No	7	9.1	70	90.9	77					
Known the usage of condoms										
Yes	4	7.5	49	92.5	53	0.03	0.87	0.90	0.24	3.35
No	6	8.3	66	91.7	72					

Table 5. Contd.

Partner working										
Local	7	10.1	62	89.9	69	0.04	0.85	0.85	0.16	4.50
Mofffusal	2	11.8	15	88.2	17					
Foreign	-	-	-	-	-					
Hospitalization										
Yes	1	4.2	23	95.8	24	0.59	0.44	0.44	0.05	3.69
No	9	8.9	92	91.1	101					
Extra marital sex										
Yes	2	11.1	16	88.9	18	0.01	0.92	1.09	0.21	5.76
No	7	10.3	61	89.7	68					
Pre-marital sex										
Yes	1	7.7	12	92.3	13	0.00	0.97	0.95	0.11	8.19
No	9	8	103	92	112					
Any contraceptive methods										
Copper-T	2	11.1	16	88.9	18	6.87	0.08	-	-	-
Pills	1	3	32	97	33					
Condoms	4	28.6	10	71.4	14					
Others	2	9.5	19	90.5	21					
Use of anti -fungal/antibacterial agents										
Yes	3	6.5	43	93.5	46	0.22	0.64	0.72	0.18	2.92
No	7	8.9	72	91.1	79					
Smell/curd like vaginal discharge										
Yes	6	11.1	48	88.9	54	1.25	0.26	2.09	0.56	7.82
No	4	5.6	67	94.4	71					
Vaginal lesions/sores in genitalia										
Yes	4	11.7	30	88.2	34	0.90	0.34	1.89	0.50	7.16
No	6	6.6	85	93.4	91					
Burning micturination										
Yes	6	10	54	0	60	0.63	0.43	1.69	0.45	6.32
No	4	6.2	61	93.8	65					

Table 5. Contd.

Pain in lower abdomen										
Yes	5	9.4	48	90.6	53	0.26	0.61	1.40	0.38	5.09
No	5	6.9	67	93.1	72					
Itching										
Yes	4	8.3	44	91.7	48	0.01	0.91	1.08	0.29	4.03
No	6	7.8	71	92.2	77					
Dysuria/vulval burning										
Yes	5	9.1	50	90.9	55	0.16	0.69	1.30	0.36	4.74
No	5	7.1	65	92.9	70					

Table 6. Susceptibility pattern of *Candida albicans* isolates and non *albicans Candida* isolates.

Antifungal agent	Disc potency (mcg)	*C. albicans*		Non *albicans Candida*	
		Sensitive isolates	Resistant isolates	Sensitive isolates	Resistant isolates
		No. (%)	No. (%)	No. (%)	No. (%)
Amphotericin B	20	16 (88.8)	2 (11.2)	10 (100)	0 (0)
Nystatin	50	5 (27.8)	13 (72.2)	0 (0)	10 (100)
Ketoconazole	10	5 (27.8)	13 (72.2)	10 (100)	0 (0)
Itraconazole	30	0 (0)	18 (100)	0 (0)	10 (100)
Fluconazole	10	4 (22.2)	14 (77.8)	3 (30)	7 (70)
Clotrimazole	10	0 (0)	18 (100)	0 (0)	10 (100)

Table 7. Sensitivity/resistant pattern of *Candida albicans* isolates.

S/N	Name of the antibiotic	Zone of inhibition			
		Minimum	Maximum	Mean	SEM
1	Amphotericin B	11	30	20.5	9.5
2	Nystatin	11	28	19.5	8.5
3	Ketoconazole	11	30	20.5	9.5
4	Itraconazole	11	16	13.5	2.5
5	Fluconazole	13	31	22	9.0
6	Clotrimazole	10	16	13	3.0

Table 8. Sensitivity/resistant pattern of non *albicans Candida* isolates.

S/N	Name of the antibiotic	Zone of inhibition			
		Minimum	Maximum	Mean	SEM
1	Amphotericin B	14	20	17	3.0
2	Nystatin	10	18	14	4.0
3	Ketoconazole	20	32	26	6.0
4	Itraconazole	10	14	12	2.0
5	Fluconazole	13	37	25	12.0
6	Clotrimazole	13	17	15	2.0

found by Saikia et al. (2009), who reported a prevalence of 21.5% in patients attending VCTC and STD clinic in Assam. Mohanty et al. (2007) reported 18.5% prevalence of vulvovaginal candidiasis in a community setting. Among the isolates, *C. albicans* was the most frequently isolated species with 14.4% and the prevalence of non *albicans Candida* was 8%. Srinivasan and Kenneth (2005) in Southern India also reported that *C. albicans* was the frequent species isolated from clinical specimens. The result was also supported by the findings of Fadda et al. (2008) that *C. albicans* is the species most commonly isolated from clinical materials although infections with other species of *Candida* have been described in recent times. In the present study, the prevalence was found to be higher in the age group of 16 to 35 years. Khan and Khan (2004) reported that *C. albicans* was the only yeast-like fungi found in the age group 15 to 30 (6.6%) and in age group 31 to 45 with frequency of 1.6%.

The outcome of the study also revealed that majority of the vulvovaginal candidiasis was observed in illiterates and married women. It was similar to the observation by Jindal et al. (2010) who conducted their study at Punjab reported that majority of the infection occurs in illiterates or had education up till primary level and were married off. Nearly 75% of the Candidal infections were observed in people of low socio-economic status and moderate economy with evidence of the work done by Ray et al. (2008) that majority of the RTI infections occur in illiterate women and low socio-economic status. Regarding the occupation, women gone as daily wagers were highly infected than the house wives (72.2% in *C. albicans* and 80% in non *albicans Candida*). The result was strengthened by Oviasogie and Okungbowa (2009) and stated that full time housewives had the lowest prevalence when compared to others. The knowledge about STDs was very less in the positive subjects and the variables like premarital and extramarital sex was not significantly associated. Jaswal and Harpham (1997) reported that when conducting research in India they found a culture of silence surrounding all gynecological conditions. Bhopal (1995) stated that Asian families are one of the most closed and private of all social groups. Even, after belonging to the low socio-economic status and illiteracy, majority of the positive subjects knew about the usage of condoms. This was attained by the efforts and suggestions given by health care personnel. In various contraceptive methods, majority of the positive subjects use the condoms. This has been controversy with the results revealed by Enweani et al. (2001). They concluded that there was a significant association between the use of contraceptive pills and vaginal colonization with *Candida* species. In the present study, there was no significant association between the place of partner working and hospitalization with the vulvovaginal candidiasis.

The use of antibacterial and antifungal agents influences the positivity only in *C. albicans* and not in non *albicans Candida* species. It was concluded by Xu et al. (2007) that short course of oral antibiotics was associated with increased prevalence of positive *Candida* colonization. The presence of symptom in positive subjects was frequently associated with positivity but statistically not significant. The commonest complication for *C. albicans* was Dysuria followed by smell and curd like vaginal discharge, burning micturition, pain in lower abdomen, itching and vaginal lesions or sores in genitalia. In non *albicans Candida* species, smell and curd likevaginal discharge and burning micturition dominates. These symptoms were recorded in the present study and similarly explained by Rao et al. *(*2006) also the same symptoms of reproductive tract infections.

Anti-fungal susceptibility testing was performed for both *C. albicans* and non *albicans Candida* species with Amphotericin B, Nystatin, Ketoconazole, Itraconazole and Clotrimazole. The resistance to Amphotericin B is infrequent for both *Candida* species. Fluconazole and Nystatin were effective on only 25% of the isolates. All the *C. albicans* strains were resistant towards Itraconazole and Clotrimazole. Non *albicans Candida* was susceptible only to Amphotericin B and Ketoconazole and resistant to all other antifungals used. The study conducted by Gross et al. (2007) revealed that resistant developed against Miconazole was the most common and all other *Candida* strains were sensitive to Fluconazole, Itraconazole and Ketoconazole.

Conclusion

Effective antifungal treatment is an important criterion in treating the candidial infections. Therefore, screening programme is essential to monitor the antimicrobial resistance and new strategies should be insisted to maximize the benefit and prolonged use of antifungals. The basic information on the epidemiology of Candidiasis plays an important role in these control strategies. This study also provides the baseline information on the prevalence and antifungal susceptibility pattern of *Candida* isolates and further research with large sample sizes is required to know the original data regarding the prevalence and their socio-demographic profiles.

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