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Yeast Flora of the Human Vagina and Effects of Antifungal Agents on its Growth *in vitro*

Mamdouh S.A. Haridy* and Moustafa, A[1]

Botany Department, Faculty of Science, Gynaecology Department,
[1]Faculty of Medicine, El-Minia University, El-Minia, Egypt.

ABSTRACT: Yeast strains were isolated from healthy women(36 isolates), infertile women(15 isolates) and women suffering from local morbidity(82 isolates). On the basis of 37 different physiological and morphological characteristics, the isolated 133 yeast strains were assigned to 10 species belonging to 5 genera. Four pathogenic species were identified. They were *Candida albicans, Candida parapsilosis, Candida tropicalis* and *Trichosporon beigelii. Candida albicans* was the dominant species, whereas *Saccharomyces cerevisiae* provailed among the saprophytic species. The percentage occurrence as well as the pattern of yeast species differed in the diagnostic groups. It was higher in the women suffering from local morbidity than in the healthy and infertile women. Moreover, a wider spectrum of species was isolated from this group. Women with intrauterine contraceptive devices showed the highest percentage of yeast occurrence which reached 50% of those tested. Five different antifungal agents were tested fro their effects on the growth of the isolated yeast species in vitro. Nystatin was the most effective against the isolated yeast species, followed by pyrithion zink and ciclopiroxolamine, whereas micronazole and clotrimazole, showed a lesser effect.

KEYWORDS: Yeast flora, vagina, antifungal agents.

Body surfaces and cavities open to the external environment are colonised by microbes soon after birth(Tannock, 1988). Since the introduction of antibiotics, steroids, antimitotic and immunsuppresive drugs, yeasts have become a major group amongst the opportunistic pathogens(Haley, 19 71 ; Adams & Cooper, 1974 ; Dolan, 1974 ; Fleisher, 1974). Among the reasons for the increase in opportunistic infections are conditions such as cancer, diabetes, severe burns or open wounds which occur in seriously ill or injured patients (Dolan, 1974 ; Louria, 1974 ; Hurley, 1980).

The important yeast pathogens of humans and animals are classified as imperfect fungi. Of fifteen genera of Cryptococcaceae, five(*Candida, Cryptococcus, Malassezia, Rhodotorula Trichosporon*) include species pathogenic to humans and animals(Gentles and Touche, 1969 ; Hurley, 1980, Hurley *et al.*, 1987).

The superficial Candida mycoses constitute a real public health hazard and prove costly in terms of medical expertise and of money spent on treatment that is often ineffective(Hurley, 1980). No study has been performed previously in Egypt. We report on the yeast flora of the human vagina in patients from El-Minia city as part of a general survey of yeast pathogens of humans in Egypt. We also report on the effects of some antifungal agents on the growth of the isolated yeast species in vitro.

Materials and Methods

I-Collection of samples : Swabs were taken from vulvar, vaginal and cervical epithelium. The patients came from private clinics and general hospitals at El-Minia city. Three groups of women were selected. The first group of women were asymptomic, whereas the second group had primary or secondary infertility. The third group had local vaginal morbidity. Swabs were directly streaked on two sets of plates containing yeast malt

*Corresponding author

Table 1. Percentage recovery of yeasts from the human vagina.

Diagnostic group	Number of women tested	Percentage recovery of yeasts
Healthy		
pregnant	100	18
non pregnant	200	9
Local morbidity		
pregnant	100	22
non pregnant	400	15
Primary of secondary infertility	100	15

agar medium adjusted to pH 3.5(Lodder, 1970) and then transferred to laboratory.

II-Isolation and identification of yeast strains : One set of the inoculated plates was incubated at 28℃, whereas the other set was incubated at 37℃. The incubation period was 2-3 days. The developing yeast colonies were examined during the period of 2-3 days. The developing yeast colonies were examined microscopically, purified, preserved on yeast-malt agar slants and stored at 4℃. Identification of the isolated yeast strains was performed according to Lodder(1970), Barnett *et al.*,(1983) and Kreger van Rij(1984).

III-Effects of some antifungal agents on the growth of the isolated yeast species in vitiro : Three different types of antifungal agents were selected to be tested for their effects on the growth of the isolated yeast species(Table 4). Drugs of the polyene(nystatin) and imidazole(miconazole and clotrimazole) classes are widely used in the treatment of yeast infections. Those of the third group (pyrithion zink and ciclopirox-olamine) are generally used in the treatment of fungal infections. Miconazole, nystatin and ciclopirox-olamine were firstly dissolved in dimethyl sulfoxide, whereas clotrimazole and pyrithion zink were directly incorporated into sterilized cultivation medium before its solidification. Definite weight(0.01-1 gm/*l*) of each antifungal agent was mixed with sterilized yeast malt agar medium(Lodder, 1970) adjusted to pH 7. Plates were then inoculated by fresh cultures(24-48 hrs). A loopful of cultured yeast species was streaked on the plates, incubated at 37℃ for 3-5 days and examined visually for growth of yeasts.

Results and Discussion

Table 1 shows the percentage of yeasts recovered from the human vagina in the three groups studied. It was clear from Table 1 that the percentage of yeast recovery was generally higher in pregnant women than in those who were not pregnant. Moreover, the pernentage was relatively higher in those pregnant women with local morbidity than in those pregnant but otherwise healthy. Non pregnant women suffering from local morbidity also showed a higher rate of yeast infections than healthy non pregnant women. Similar obsevations were reported by Odds(1979), Hurley(1980) and Hurley *et al.*,(1987). The percentage of yeast infections in infertile women reached 15%. Further work should be planned to throw light on the relationship between infertility and yeast infection.

It was of interest that non pregnant women with intrauterine contraceptive device showed a high rate of yeast infection(50% of the women tested). Moreover, women in the third decade of age showed a higher percentage of yeast infection(23%) than in the second decade of age(5%). Hurley (1980) and Hurley *et al.*,(1987) commented that there is a tendency for yeast colonization to increase with age of the patient, particularly in the case of oral cavity.

Table 2 shows the distribtuion of the isolated yeast species in relation to diagnostic group. A wider spectrum of yeast species was isolated from women suffering from local morbidity than from healthy and infertile women. Moreover, *Candida albicans* was the most dominant species and was represented by 82% of the isolates. Its isolation from healthy and infertile women was obviously lower and represented 53% and 13% of the isolation respectively. *Candida parapsilosis* was the most dominant species in infertile women. *Can-*

Table 2. Distribution of the isolated yeast species by the diagnostic group.

Yeast species	Total number of isolates(133)	Diagnostic groups		
		healthy	local morbidity	primary of secondary infertility
*Candida albicans**	88	19	67	2
*Candida parapsilosis**	16	7	4	5
*Candida tropicalis**	9	6	3	–
Saccharomyces cerevisiae	9	2	3	4
Candida intermedia	4	–	1	3
*Trichosporon beiglii**	2	2	–	–
Pichia guilliermondii	2	–	2	–
Candida conglobata	1	–	–	1
Candida melibiosica	1	–	1	–
Hansenula anomala	1	–	1	–

* = Pathogenic yeast species

dida tropicalis was represented by a considerable number of strains and was isolated only from healthy women and women suffering from local morbidity. *Trichosporon beigelii*, another pathogenic yeast species, was isolated only from healthy women. Generally, *Candida albocans* was the dominant species of the yeast flora of both healthy women and of those suffering from local morbidity. This observation was also reported by Hurly *et al.*,(1973) and Odds(1979). Of interest was the isolation of three pathogenic yeast species other than *Candida albicans* in this study. These species were *Candida parapsilosis, Candida tropicalis* and *Trichosporon beigelii*. The isolation of pathogenic yeast species other than *Candida albicans* may predicate a serious fungal infection as reported by Hurley *et al.*,(1987).

Table 3 shows physiological and morphological properties of the isolated yeast species. On the basis of 37 physiological and morphological characteristics, isolated strains were assigned to 10 species belonging to 5 genera. *Candida albicans* was the most dominant species. Three pathogenic yeast species other than *Candida albicans* were identified. These species were *Candida Parapsilosis, Candida tropicalis* and *Trichosporon beigelii*. These pathogenic species were differentiated from each other by glucose fermentation, arabinose, lactose, raffinose, arabinitol, gluconate and lactate assimilation, growth at 42℃, demonstration of true mycelium, chlamydospores and arthrospores and formation of germ tubes(Table 3).

Table 4 shows the effects of 3 different groups of antifungal agents on the growth of the isolated yeast species. Nystatin, a member of the polyene group, inhibited growth of the isolated yeast species at concentration ranging from 0.01 to 0.02 gm/*l*. One notes that the pathogenic *Candida* species were more sensitive to nystatin than was the pathogenic species *Trichosporon beigelii*(Table 4). Hurley *et al.*,(1987) reported that nystatin was inhibitory to *Candida albicans* and *Candida glabrata* at concentration ranging from 0.0004 to 0.001 gm/*l*. Kitahara *et al.*,(1976) reported that inoculum size, termperature, duration of incubation and medium composition effect the minimal inhibitory concentrations of polyenes. Generally, nystatin was the most effective antifungal agent against yeasts. Miconazole and clotrimazole members of the imidazoles, showed an inhibitory action against the isolated yeast species at concentration ranging from 0.6 to 1.0 gm/*l* and 0.3 to 0.5 gm/*l* respectively. It is clear from Table 4 that clotrimazole is superior to miconazole in its action on the gro-

Table 3. Physiological and morphological properties of the isolated yeast species.

Yeast species	Fermentation															Assimilation																		Growth at		Demonstration of				
	Number of strains tested(133)	glucose	galactose	maltose	sucrose	lactose	galactose	sorbose	ribose	xylose	arabinose	rhamnose	sucrose	maltose	trehalose	melibiose	lactose	raffinose	melezitose	erythritol	ribitol	arabinitol	mannitol	galactitol	inositol	gluconate	lactate	succinate	citrate	37℃	42℃	ascospores	true mycelium	pseudomycelium	pellicle	germ tube	chlamydospores	arthrospores
*C. albicans**	88	100+	100	100	0	0	100	91	82	100	64	0	100	100	91	0	0	0	100	0	91	0	100	0	0	100	100	100	100	100	100	0	100	100	100	100	100	0
*C. parapsilosis**	16	100	100	50	25	0	100	100	75	100	100	0	100	100	100	0	0	0	100	0	100	50	100	0	0	0	0	100	100	100	100	0	0	100	100	0	0	0
*C. tropicalis**	9	100	100	100	100	0	100	100	33	100	0	0	100	100	100	0	0	0	100	0	100	0	100	0	0	100	100	100	100	100	100	0	100	100	100	0	0	0
S. cerevisiae	9	100	11	11	11	0	67	0	0	0	0	0	67	67	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	100	100	100	0	67	67	0	0	0
C. intermedia	4	100	100	75	75	0	100	100	0	100	0	0	100	100	100	0	100	100	100	0	100	0	100	0	0	50	0	100	100	100	0	0	0	100	100	0	0	0
*T. beigelii**	2	0	0	0	0	0	100	100	100	100	0	0	100	100	100	0	100	100	100	0	100	100	100	0	0	100	100	100	100	100	0	0	100	100	100	0	0	100
P. quilliermondii	2	100	0	0	0	0	100	100	0	100	0	0	100	100	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	100	50	100	0	100	100	0	0	0
C. conglobata	1	100	0	0	0	0	100	100	0	100	100	0	0	0	100	0	0	0	0	100	100	100	100	0	0	100	0	100	0	0	0	0	0	100	100	0	0	0
C. melibiosica	1	100	100	0	0	0	100	100	100	100	100	0	100	100	100	100	0	100	100	0	100	0	100	0	0	100	0	100	100	100	100	0	0	100	100	0	0	0
H. anomala	1	100	100	0	100	0	100	100	0	100	0	0	100	100	100	0	0	100	100	0	100	0	100	0	0	100	100	100	100	100	0	100	0	100	100	0	0	0

* = Pathogenic yeast species *C.* = *Candida* *H.* = *Hansenula* *P.* = *Pichia* *S.* = *Saccharomyces* *T.* = *Trichosporon*
+ = Percentage of positive reactions of strains

Table 4. Effects of some antifungal agents on the growth of isolated yeast species *in vitro*.

	Minimal inhibitory concentration(gm/*l*)									
	*C. albicans**	*C. parapsilosis**	*C. tropicalis**	*T. beigelii**	*S. cerevisiae*	*C. intermedia*	*P. guilliermondii*	*C. conglobata*	*C. melibiosica*	*H. anomala*
Number of the isolates tested	20	10	9	2	9	4	2	1	1	1
Antifungal agents :										
a−Polyene compounds :										
nystatin(nystaform, cream)	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01
b−Imidazole compounds :										
miconazole(gyno-daktarin, cream)	1.00	0.9	0.9	0.6	0.6	0.8	0.6	0.8	0.8	0.6
clotrimazole(dermatin, solution)	0.5	0.5	0.5	0.3	0.3	0.5	0.3	0.5	0.5	0.3
b−Other antifungal compounds :										
pyrithion zink(de-squaman, cream)	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.02	0.02
clotrimazole(dermatin, solution)	0.05	0.05	0.05	0.04	0.04	0.05	0.04	0.05	0.05	0.05

* =Pathogenic species *C.*=*Candida* *T.*=*Trichosporon* *S.*=*Saccharomyces* *P.*=*Pichia* *H.*=*Hansenula*

wth of the isolated species. Mahgoub(1972) and Drouhet(1973) reported that clotrimazole and miconazole are inhibitory to all fungi of medical importance at a concentration of 10 mg/*l*. Two wide variation in imidazole activity noted in the published reports is probably due to differences in the methodology used as suggested by Hurley *et al.*, (1987).

The effects of the third group of antifungal agents to which pyrithion zink and ciclopirox-olamine belong were of interest. These compounds were inhibitory to the isolated yeast species. Their minimal inhibitory concentrations ranged from 0.02 to 0.04 gm/*l* in the case of pyrithion zink and from 0.04 to 0.05 gm/*l* in the case of ciclopiroxolamin (Table 4). According to their minimal inhibitory concentrations, they were superior to the tested imidazole compounds. Further work should be planned to study their application in the treatment of yeast infections. Table 4 shows that *Trichosporon beigelii* tolerated a two fold higher concentration of pyrithion zink than the other pathogenic yeast species. Therefore, pyrithion zink containing medium can be used as specific medium for the isolation of *Trichosporon beigelii*.

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