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Airborne Yeast Isolates as Biocontaminants at Two Different Indoor Environments in Cairo

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ABSTRACT

This study was conducted during the period of November 2000 to October 2001 to trap, enumerate and identify the different airborne yeast isolates in a variety of microhabitats of indoor environments in two different localities in Cairo (Opera house & Police prison rooms). The settle plate method was used on different media. A total of 10 genera and 25 species were trapped from all exposures. The total yeast catched (in cfu) obtained from all exposures during the four seasons were 860 colonies. Police prison rooms count recorded the highest count (640 cfu) than Opera house (256 cfu). The genus *Candida* was the dominate genus representing 74.4% of the total count. Highest count was recorded in autumn (546 cfu) and the lowest in spring (59 cfu). The isolated yeasts were tested for their ability to produce mycotoxin by studying their effects on Gram-positive bacteria (*B. subtilis*), Gram-negative bacteria *(E. coli)*, yeast (*C. albicans*) and mold fungi (*A. niger*). Many isolates produced antimicrobial toxins in variable amounts *Candida steatolytic* and *Rhodotorula glutinis* have antagonistic activity against the four tested human pathogens. The pathogenic potentialities of some selected *Candida* species were studied. They were manifested by growth at 37^0C, production of proteolytic and lipolytic enzymes and blood haemolysis. Enzyme assay for the proteolytic enzymes (acidic, neutral & alkaline proteases) were studied. The different *Candida* species selected yielded variable amounts of proteolytic enzymes and acid proteases were produced in relatively high amounts. *Candida albicans* recorded the maximum yield of acid protease. Antifungal sensitivity test of selected *Candida* species revealed that they were sensitive to Nystatin and varied towards the other antifungal agents.

Key Words: Yeast isolates; Biocontaminants; Environment; Human health; Cairo

INTRODUCTION

It has been found that higher isolates of *Trichosporn custaneum*, total yeasts, and total fungi were associated with unsanitary rooms, and especially with damp places in the wood or tatami mats in patient's homes. No recurrence was observed in 6 well cleaned homes, but recurrence was observed in all patients who resided in homes that were not cleaned or in homes where cleaning was not adequate and where *T. cutaneum* was not eliminated (Yoshida *et al.*, 1989).

Prevention of airborne invasive fungal infection of people suffering from immunodeficiency mainly concerns the control of fungal spores in indoor air. The environment and morphogenesis of infective particles of *Aspergillus, Mucoraceae, Cryptococcus neoformans* and some other yeast-like fungi are of main interest (Staib, 1992).

In the evolution of distribution of mold genus over a period of one year, *Cladosporium* and *Pencillium* make up 88% identified colonies and constantly present in considerable concentration, with a decrease during winter months for *Cladosporium* and an increase from November to April for *Penicillium*. *Alternaria* was detected particularly in September and October; *Aspergillus* is constantly present in low conc. *Candida* and *Mucor* were detected only occasionally (Di Berardino *et al.*, 1990).

It was found that at low altitude closed rooms provided lower concentration of potential fungal allergens than outdoor environment. On the other hand, at high altitude, it showed more than double the concentration of fungal spores than outdoor the nearby outdoor atmosphere. In all indoor environments the spore count decrease in the order of *Cladosporium* >yeast >mycelia sterilia >*Penicillium* >*Epicoccum* >*Botyris* >*Aureobasidium* >*Alternaria*. The total airborne propagules of *Cladosporium*, mycelia sterilia, *Alternaria*, *Botyris* and *Epicoccum* showed season distribution (Ebner *et al*., 1992).

Human dwellings are seldom sources of invasive mycotic disease in immunocompetent persons but may harbour molds and yeasts capable of causing opportunistic infection and allergenically mediated diseases (Summerbell *et al*., 1992). Fungi present in work-related, recreational or living environment can cause several diseases (Kurup *et al*., 1984). These diseases may be invasive, the result of exposure to establish pathogens or they may be opportunistic infections and allergies due to saprophytic molds of special interest are the widespread of mycotic diseases in heavy agro-industries. Opportunistic pathogens such as *Aspergillus, Penicillium*, *Fusarium*, *Geotrichum* and yeasts such as *Candida albicans* were detected in patient's sputa, sera and isolated from grains, bagasse and indoor air (Kurup *et al*., 1984).

Nosocomial fugal infection is usually correlated with two opportunistic genera *Candida* and *Aspergillus*. It has been reported that *Candida* is now the fourth most frequently reported pathogen associated with nosocomial "blood searum infection" and in "Intensive Care Units" (Jarvis, 1991). Rantala (1993) reported that *Candida* species are important opportunistic pathogens in compromised hosts such as patients recovering from major abdominal surgery. Postoperative candidiasis was most often encountered in patients undergoing surgery of the small intestine and pancreas. These patients have certain typical features, central catheterization, parental nutrition, prolonged antibiotic therapy and re-operations.

Cvetnic and Pepeljnjak (2001) counted approximately 954 fungal colonies and 980 yeast colonies from indoor environment and stated that the spores of these fungi are known to be allergic. Su *et al.* (2001) reported that the prominent genera of indoor airborne fungi were *Cladosporium, Aspergillus, Penicillium, Alternaria* and yeast. In addition, seasonal effects seem to be domestic endotoxin in study homes. Ismail *et al.* (2000) reported that yeasts were among the most prevalent fungi from both indoor and outdoor microhabitats.

MATERIALS AND METHODS

Isolation enumeration and identification of indoor airborne viable yeast isolates. The gravity setting culture plate collections method was used to isolate yeasts for the quantitative and qualitative study in two environments area in Cairo (Opera house (O) and Police prison rooms (P)) over a period of 12 months, from November 2000 to October 2001. Sampling was carried out monthly at different sites. From Opera house isolation sites include (i) Big theatre, (ii) Small theatre, (iii) Hanager theatre, (iv) Coffee, (v) Bathrooms, (vi) Music library and (vii) Training rooms. Police prison room's isolation sites include men and women prison rooms of Shoubra and Azbakya police buildings.

Sampling and identification of indoor yeast isolates. Sampling was made at 1 meter above the ground level and the culture plates were exposed for about 30 minutes, then the plates were transferred immediately to the laboratory and incubated for 15 days at 28^0C. Colony counts were carried out and observed daily. Media used were Sabouraud's glucose agar "g/L" (glucose, 20; neopeptone, 10; agar, 20; distilled water, 1 litre & chloramphenicol 0.05) and Wickerham's agar media "g/L" (glucose, 10; peptone, 5; yeast extract, 3; malt extract, 3; agar, 20; distilled water, 1 litre). All the media used were supplemented with antibiotic to eliminate bacterial contamination (Wickerham, 1951). All developing colonies of yeast isolates were further isolated on agar slants from representative plates for subsequent identification.

Species count, dominance and frequency of indoor yeast isolates. Species counts were expressed as absolute total number cfu (colony forming units) monthly recorded throughout the experimental period (12 months). Species dominance was expressed as percentage number of cfu of each species in relation to the total count of all species recorded through the experimental period. Species frequency was expressed as percentage number of cases of isolation of each species out of the total expressed plates.

Identification of yeast isolates. Yeast isolates were identified to the genus and species level by carrying out the following steps: morphology on corn–meal tween–80 agar medium, growth at different temperature, cycloheximide sensitivity test, starch test, urease test, gelatine liquefaction test, biochemical tests (fermentation & assimilation of carbohydrates), utilization of nitrogen compounds and nirite assimilation test (Wickerham, 1951; Barnett, 1966; Lodder, 1970, 1971; Skinner *et al*., 1980; Arx, 1981; Barnett *et al.*, 1983; Kreger, 1984; Barnett *et al*., 1990).

Screening of mycotoxine producing ability of yeasts isolated from indoor air of Opera house (O) and Police prison rooms (P). All yeast isolates were inoculated on Czapek's dox agar plates and incubated for 7 days at 28^0C. Disks 5 mm in diameter were transferred to dox agar plates inoculated with *Bacillus subtilis, Escherichia coli, Candida albicans* and *Aspergillus niger* and incubated at 37^0C for 24 h, the clear zone around each disk was measured (Clements, 1968).

Physiological characteristics of some selected pathogenic *Candida* species isolated from indoor air of Opera house (O) and Police prison rooms (P). Seven *Candida* species isolated from Opera house (O) and Police prison rooms (P), namely *Candida albicans, C. guilliermondii, C. krusei, C. parapsillosis, C. pseudotropicalis, C. steatolytica* and *C. tropicalis* were selected and the following tests were carried out:

1. **Temperature tolerance.** Two tube of Sabouraud's dextrose agar were inoculated with the test orgnaism and incubated at 25^0C and 37^0C for 7-14 days.
2. **Blood haemolysis.** Two plates of blood agar were inoculated with the test organism and incubated at 25^0C and 37^0C for 7-14 days.
3. **Production of proteolytic enzymes.** Detection of proteolytic enzyme activity: The solidified medium used for the detection of proteolytic enzyme activity "g/L" (glucose, 10; agar, 20; casein, 5; yeast extract, 3; malt extract, 3; distilled water, 1 litre) was streaked with a pure colony of *Candida* species and incubated at 28^oC for 2-14 days. The plates were then flooded with 8-10 mL of reagent (mercuric chloride, 15 gm; conc. HCL, 20 mL; distilled water, 100 mL). Unhydrolysed casein produced a white opaque precipitate while hydrolysed casein appeared as a clear zone.

Quantitative determination of proteolytic enzymes activity. Yeast extract dextrose medium "YED" "g/L" (yeast extrat, 10; casein or skimmed milk, 20; glucose, 20; dist. water, 1 L) was inoculated with the tested *Candida* species 48 h cultures then inoculated into flask containing 50 mL of YED broth medium supplemented with Casein or

skimmed milk (the inoculum volume was 10^8cfu/mL yeast). The inoculated flasks were shaked at 150 rpm at 37^0C for 72 h. Every 12 h 1 mL sample was withdrawn, centrifuged at 3000 rpm for 10 mm.

Enzyme assay. Assay Medium: 0.1. mL sodium citrate buffer containing 2 gm BSA/litre and the pH adjusted at 3.5, 6.5 and 8.5. Culture supernatant, assay medium were kept on ice. Reaction starts by adding 0.1 mL supernatant + 0.9 mL assay medium. Rapidly shaking at 37^0C for 10 min. Add equal volume of 5% trichloroacetic acid. After an additional 10 min. the reaction mixture was centrifuged. The supernatant was decanted, and the absorbance at 280 nm was read against blank containing distilled water. Enzyme units are expressed as the amount of tyrosine in micromoles released per minute per millilitre of culture supernatent (10^8 cells of *Candida* species). At the same time of enzyme assay, the growth rate was determined (Absorbance at 660 nm).

4. **Detection of lipolytic enzyme activity.** Procedure: poured and solidified plates of the medium "g/L" (malt extract, 3; yeast extract, 3; peptone, 5; butter-fat or olive oil, 50; agar, 20; dist. water, 1litre) were streaked with a pure colony of the selected *Candida* species then incubated at 28^0C for 2-14 days. The plates were then flooded with 8-10 mL of the developing reagent (saturated copper sulphate solution) and allowed to stand for 10-15 min., then the reagent was poured off and the plates were washed gently in running water for one minute to remove excess copper sulphate. Lipolytic activity appeared as bluish-green coloured zones due to formation of the insoluble copper salt of fatty acids formed.

Sensitivity of the selected *Candida* species against some antifungal agents. Sensitivity test of the selected *Candida* species was carried out using candifast kit (Hassan, 1994). It provides a sensitivity profile against 7 antifungal agents (Amphotericin B, Nystatin, 5-flurocytocine, Econazole, Ketoconazole, Miconazole & Fluconazole).

Statistical analysis. One way analysis of variance was carried out and from which L.S.D. was calculated to compare the different means in the various sites and seasons examined (Table I).

RESULTS

In the present investigation, it has been found that the total count in cfu (colony forming units) of indoor air of Opera house (O) and Police prison rooms (P) reached 860 cfu during the four seasons. Yeasts isolated from (O) constituted a low total count (256 cfu) compared with that isolated from (P), (604 cfu). It was found that the highest count was recorded in autumn (546 cfu) and the least was in spring (59 cfu) (Table II; Fig. 1). Concerning the site of isolation, yeast count ranged from 500 cfu from (P) in autumn to 19 cfu from (O) in spring (Table II; Fig. 2).

Yeasts were identified on the bases of their morphological, physiological and biochemical characteristics. Seasonal count, frequency and percentage of the different indoor yeast species isolated from Opera house (O) and Police prison rooms (P) during the four seasons were shown in Table III. Results showed that total number of yeast species ranged from 15 species isolated in autumn (represented by 546 cfu), 17 in winter (represented by 188 cfu), 15 in spring (represented by 59 cfu) and 11 in summer (represented by 67 cfu) (Table II & III). In this study 25 yeast species were isolated belonging to 10 genera, namely, *Candida, Cryptococcus, Debaromyces, Endomyces, Geotrichum, Kloechera, Rhodotorula, Saccharomyces, Torulopsis* and *Trichosporon* (Table III).

From Opera house, the number of yeast species isolated during autumn was 10 species and the common species recorded were *Candida guilliermondii* (represented by 14 isolates) and *Debaromyces hansenii* (represented by 11 isolates) followed by *Endomyces fibuliger* (represented by 6 isolates) and *Crypococcus albidus* (represented by 5 isolates). The rest of species were less common (Table IV). In winter 15 yeast species were isolated of which *Endomyces fibuliger* (represented by 52 isolates), *Candida tropicalis* (represented by 29 isolates) *and C. krusei* (represented by 25 isolates) were the most prevalent yeast species. 4 species of yeasts were also isolated during spring of which *Candida Catenulata* (represented by 16 isolates) is the most common species isolated, other species were of less occurrence. In summer 8 species of yeasts were isolated. Three species were of common occurrence namely, *Candida melinii* (represented by 5 isolates), *C. tropicalis* (represented by 4 isolates) and *Cryptococcus albidus* (represented by 4 isolates) (Table IV).

Yeast species isolated during this study from police

Table I. Descriptive statistics for results obtained for different species as affected by site and season. Mean standard deviation and standard error of species as affected by site and season. Difference was significant in Opera and Police at different seasons at 5% level

Season	Site							
	Opera				Police			
	Mean	St.D.	St. error	L.S.D	Mean	St.D.	St. error	L.S.D.
Autumn	1.84	3.62	1.32	1.84	20.0	63.52	6.39	20.00
Winter	6.72	12.14	1.32	6.72	0.80	1.66	6.39	0.80
Spring	0.76	3.19	1.32	0.76	1.68	2.66	6.39	1.68
Summer	0.92	1.55	1.32	0.92	1.68	6.19	6.39	1.68
Total	2.56	6.92			6.04	32.49		

Table II. Total count in cfu of yeast species isolated from Opera house (O) and Police prison rooms (P) durning the four seasons

Count in cfu								Total count in cfu	
Autumn		Winter		Spring		Summer			
O	P	O	P	O	P	O	P	O	P
46	500	168	20	19	40	23	44	256	604
546		188		59		67		860	

Table III. Seasonal count, frequency and percentage of the different indoor yeast species isolated from Opera house (O) and Police prison rooms (P)

No.	Yeast species	count												Total count cfu	
		Opera						Police house prisons						O+P	%
		Autumn	winter	Spring	summer	Total	%	Autumn	winter	Spring	summer	Total	%		
1	*Candida albicans*	-	6	-	-	6	54.5	3	2	-	-	**5**	45.5	11	1.27
2	*C. catenulata*	3	11	16	2	32	84.2	6	-	-	-	**6**	50	38	4.41
3	*C. famata*	-	-	-	-	-	0	250	-	3	-	**253**	100	253	29.41
4	*C. guilliermondii*	14	8	-	-	22	91.7	1	1	-	-	**2**	8.3	24	2.97
5	*C. krusei*	3	25	-	-	28	73.7	-	-	9	1	**10**	26.3	38	4.41
6	*C. typestrain*	-	-	-	-	-	0	-	-	-	1	**1**	100	1	0.11
7	*C. melinii*	-	-	-	5	5	2.3	210	-	1	-	**211**	97.7	216	25.11
8	*C. parapsillosis*	-	3	-	-	-3	60	2	-	-	-	**2**	40	5	0.58
9	*C. Pseudotropicalis*	-	-	-	-	-	0	-	-	2	-	**2**	100	2	0.23
10	*C. silvae*	-	-	-	-	-	0	-	-	2	-	**2**	100	2	0.23
11	*C. steatolytica*	1	1	-	-	2	100	-	-	-	-	**-**	0	2	0.23
12	*C. tropicalis*	1	29	1	4	35	72.9	2	3	5	3	**13**	27.1	48	5.63
13	*Cryptococcus albidus*	5	4	1	4	14	93.3	-	-	-	1	**1**	6.7	15	1.74
14	*Debaromyces hansenii*	11	2	-	-	13	56.5	3	7	-	-	**10**	43.5	23	2.67
15	*Endomyces fibuliger*	6	52	-	-	58	84.1	10	-	-	1	**11**	15.9	69	8.02
16	*Geotrichum candidum*	-	-	-	-	-	0	-	1	-	-	**1**	100	1	0.11
17	*Kloechera apiculata*	1	3	-	2	6	50	1	-	5	-	**6**	50	12	1.40
18	*Rhodotorula glutinis*	-	-	1	-	1	100	-	-	-	-	**-**	0	1	0.11
19	*R. rubra*	-	-	-	-	-	0	-	1	-	-	**1**	100	1	0.11
20	*Saccharomyces cereviciae*	1	5	-	3	9	39.1	8	1	1	4	**14**	60.9	23	2.67
21	*Torulopsic glabrata*	-	3	-	-	3	25	-	-	9	-	**9**	75	12	1.40
22	*Trichosporon beigelii*	-	2	-	2	4	100	-	-	-	-	**-**	0	4	0.47
23	*T. cutaneum*	-	14	-	1	15	26.8	4	4	2	31	**41**	73.2	56	6.51
24	*T. fermentans*	-	-	-	-	-	0	-	-	1	-	**1**	100	1	0.11
25	*T. penicillatum*	-	-	-	-	-	0	-	-	2	-	**2**	100	2	0.23

prison rooms are shown in Table V which shows a high total count of 500 cfu obtained during autumn followed by summer 44 cfu, spring 40 cfu and a low total count of 20 cfu was obtained during winter. Species isolated from police prison rooms are belong to genera *Candida, Cryptococcus, Debaromyces, Endomyces, Geotrichum, Kloechera, Rhodotorula, Saccharomyces, Torulopsis* and *Trichosporon* (Table VI).

Number of yeast species isolated during autumn was 12 and the common species recorded were *Candida famata* (represented by 250 isolates) and *C. melinii* (represented by 210 isolates). The rest of species were of less common. In winter 8 yeast species were isolated of which *Debaromyces hansenii* (represented by 7 isolates) is the most common species. In spring 11 yeast species were isolated of which *Candida krusei* (represented by 9 isolates), *Torulopsis glabrata* (represented by 9 isolates) were the most common yeast species isolated. In summer 8 yeast species were isolated of which *Trichosporon cutaneum* (represented by 31 isolates) is the most common yeast species isolated, the rest of species were of less occurrence.

Screening of mycotoxine producing ability of yeast fungi isolated from indoor air of Opera house (O) and Police prison rooms (P). All yeast species isolated from Opera (O) and Police (P) were examined for production of mycotoxins. The effect of mycotoxins on Gram-positive bacteria (*B.subtilis*), Gram-negative bacteria (*E.coli*), yeast (*C.albicans*) and mold (*A.niger*) were also studied. Many isolates produce antimicrobial toxins in variable amounts (Table VII). This table shows that there are three categories, the first category include only 2 yeast species *Candida steatolytica* and *Rhodotorula glutinis* which have antagonistic activity against all the four tested human pathogens. *Candida steatolytica* was the most potent antagonist of them. The second category include 6 yeast species (*Candida krusei, C. parapsillosis, C.tropicalis, Rhodotorula rubra, Saccharomyces cerevisiae* & *Trichosporon fermentans*) exhibited antagonistic activity against 3 of the 4 tested human pathogens, the third category include 3 yeast species (*C. famata, C. krusei* type strain & *C.*melinii) which have antagonistic activity against 2 of the 4 tested human pathogens and the last category include 5 yeast species (*C.albicans, C. pseudotropicalis, Endomyces fibuliger, Geotrichum candidum* & *Torulopsis glabrata*) which were found to exhibited antagonistic activity against one of the 4 tested human pathogens. The rest of yeast species have no mycotoxin production activity.

Physiological characteristics of some selected pathogenic *Candida* species isolated from indoor air of Opera house (O) and Police prison rooms (P). The pathogenic

Table IV. Count and frequency of yeast species isolated from Opera house (O) during the four seasons

Season Yeast Species	Count Autumn	Winter	Spring	Summer	Total
Candida albicans	-	6	-	-	6
C. catenulata	3	11	16	2	32
C. guilliermondii	14	8	-	-	22
C. krusei	3	25	-	-	28
C. melinii	-	-	-	5	5
C. parapsillosis	-	3	-	-	3
C. staetolytica	1	1	-	-	2
C. tropicalis	1	29	1	4	35
Cryptococcus albidus	5	4	1	4	14
Debaromyces hansenii	11	2	-	-	13
Endomyces fibuliger	6	52	-	-	58
Kloechera apiculata	1	3	-	2	6
Rhodotorula glutinis	-	-	1	-	1
Saccharomyces Cereviciae	1	5	-	3	9
Torulopsis glabrata	-	3	-	-	3
Trichosporon beigelii	-	2	-	2	4
T. cutaneum	-	14	-	1	15

Table V. Total count of yeast species isolated during the four seasons from Police (men and women prison rooms).

Total Count (cfu)								Total	
Autumn		Winter		Spring		Summer			
M.	W.	M.	W.	M.	W.	M.	W.	M.	W.
484	16	9	11	33	7	23	21	549	55
500		20		40		44		604	

potentialities of selected *Candida* species recovered from (O) and (P) were investigated by testing some of their physiological characteristics such as growth at 37^0C, blood haemolysis, gelatin liquefaction and fat-splitting. Growth of isolates of selected *Candida* species at 37^0C can indicate their ability to grow at human's body temperature (Table VIII). Similarly, *Candida* species hydrolysis, casein, gelatin and fat revealing, the production of proteolytic and lipolytic enzymes, which are implicated in the process of pathogenesis. Blood haemolysis by *Candida* species proves their invasive and dissiminated form (Table VIII).

The determination of proteolytic activity was carried out on two substrates (skimmed milk & casein) at three different pH values, 3.5, 6.5 and 8.5. The activity of enzymes and the growth rate of yeast were measured at various incubation periods.

Data obtained revealed that *Candida albicans* gave the highest yield of proteinase enzyme followed by *C. steatolytica C. pseudotropicalis* and *C. krusei* (Table IX). There was no significant difference in utilizing skimmed

Fig. 1. Mean seasonal total count in cfu of indoor yeast isolates

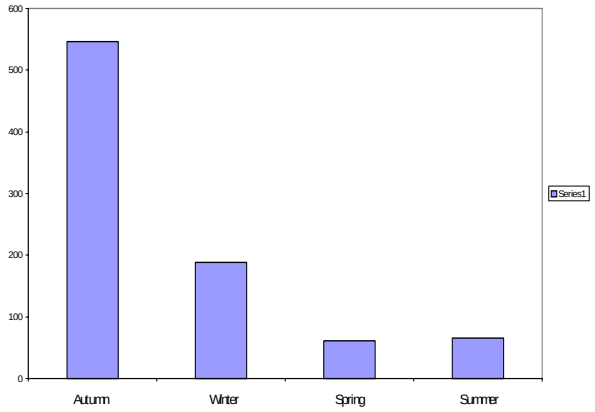
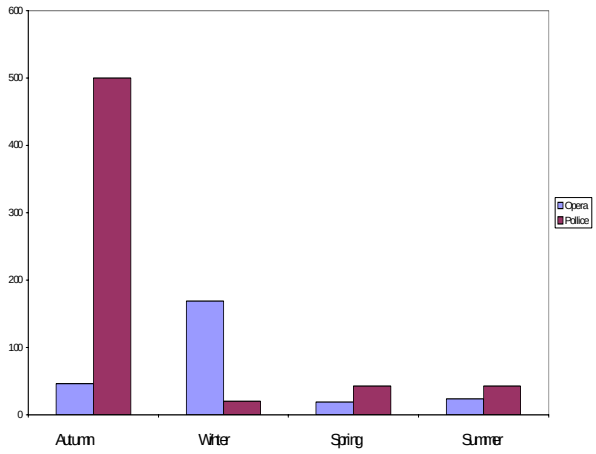


Fig. 2. Mean seasonal total count in cfu of yeasts isolated from Opera house (O) and Police prison rooms (P)



milk or casein as a substrate. Using different pH's in this test was markedly significant. The activity of proteinase enzyme in all the species tested was higher at pH 3.5 (acid proteinase) followed by (alkaline proteinase) at pH 8.5 and the least activity was obtained at pH 6.5 (neutral proteinase).

Sensitivity of the selected *Candida* species against some antifungal agents. The test show that all tested *Candida* species were sensitive to Nystatin. *Candida albicans* was also sensitive to Amphotericin B, (5-flurocytosine, ketoconazole, miconazole & fluconazole) beside Nystatin (Table X).

The reported opportunistic genera and species of yeasts isolated from Opera house and Police prison rooms were shown in Tables XI. This table showed that 17 species of yeast belonging to 7 genera were reported as opportunistic pathogens. They were *Candida, Cryptococcus, Debaromyces, Kloechera, Rhodotorula, Torulopsis* and *Trichosporon.* Concerning Opera house

Table VI. Count and frequency of yeast species isolated from Police (men and women prison rooms) during the four seasons

Season Yeast Species	Count										
	Autumn		Winter		Spring		Summer		Total		Total
	M.	W.	M.	W.	M.	W.	M.	W.	M.	W.	
Candida albicans	2	1	2	-	-	-	-	-	4	1	5
C. Catenulata	4	2	-	-	-	-	-	-	4	2	6
C. famata	250	-	-	-	3	-	-	-	253	-	253
C. guilliermondii	1	-	1	-	-	-	-	-	2	-	2
C. krusei	-	-	-	-	9	-	-	1	9	1	10
C. krusei type strain	-	-	-	-	-	-	1	-	1	-	1
C. melinii	210	-	-	-	1	-	-	-	211	-	211
C. parapsillosis	2	-	-	-	-	-	-	-	2	-	2
C. Pseudotropicalis	-	-	-	-	-	-	2	-	2	-	2
C. silvae	-	-	-	-	1	1	-	-	1	1	2
C. tropicalis	-	2	1	2	3	2	-	3	4	9	13
Cryptococcus albidus	-	-	-	-	-	-	1	-	1	-	1
Debaromyces hansenii	-	3	1	6	-	-	-	-	1	9	10
Endomyces fibuliger	6	4	-	-	-	-	1	-	7	4	11
Geotrichum candidum	-	-	1	-	-	-	-	-	1	-	1
Kloechera apiculata	1	-	-	-	5	-	-	-	6	-	6
Rhodotorula rubra	-	-	-	1	-	-	-	-	-	1	1
Saccharomyces cereviciae	4	4	1	-	-	1	3	1	8	6	14
Torulopsic glabrata	-	-	-	-	7	2	-	-	7	2	9
Trichosporon cutaneum	4	-	2	2	2	-	15	16	23	18	41
C. fermentans	-	-	-	-	-	1	-	-	-	1	1
C. penicillatum	-	-	-	-	2	-	-	-	2	-	2

Table VII. Screening for mycotoxin producing ability of yeast species isolated during the four seasons from Opera house (O) and Police prison rooms (P)

No.	Yeast species	Inhibition zone (mm)			
		B. subtilis	***E. coli***	***C. albicans***	***A. niger***
1	*Candida albicans*	-	-	-	18
2	*C. Catenulata*	-	-	-	-
3	*C. famata*	-	7	-	12
4	*C. guilliermondii*	-	-	-	-
5	*C. krusei*	9	-	8	15
6	*C. typestrain*	-	-	13	12
7	*C. melinii*	-	8	7	-
8	*C. parapsillosis*	11	-	8	15
9	*C. Pseudotropicalis*	8	-	-	-
10	*C. silvae*	-	-	-	-
11	*C. steatolytica*	10	21	8	8
12	*C. tropicalis*	10	-	8	20
13	*Cryptococcus albidus*	-	-	-	-
14	*Debaromyces hansenii*	-	-	-	-
15	*Endomyces fibuliger*	-	-	-	12
16	*Geotrichum candidum*	-	-	-	20
17	*Kloechera apiculata*	-	-	-	-
18	*Rhodotorula glutinis*	8	8	9	8
19	*R. rubra*	11	-	8	16
20	*Saccharomyces cereviciae*	-	7	8	12
21	*Torulopsis glabrata*	-	-	-	18
22	*Trichosporon beigelii*	-	-	-	-
23	*T. cutaneum*	-	-	-	-
24	*T. fermentans*	11	8	13	-
25	*T. penicillatum*	-	-	-	-

Candida catenulate, C. tropicalis and *Cryptococcus albidus* were recorded in all seasons, and *Kloechera apiculata* was recorded in three seasons. While on police house prisons *Candida tropicalis* and *Trichosporon cutaneum* were recorded in all seasons. *Candida tropicalis* is the only yeast species record in all seasons of both Opera house and police prison rooms. In Table XII a list of different mycotic diseases which associated with the different yeast species as reported by many investigator (Mc Ginnis, 1980; Rippon, 1982).

Table VIII. Physiological characteristics of the selected pathogenic *Candida* species

Candida species	Growth at 37^0C	Blood haemolysis	Fat splitting	Gelatin Liquefaction	Casein hydrolysis
Candida albicans	+	++	+++	++	++
C. guilliermondii	+	+	+	+	+
C. krusei	+	++	++	+	+
C. parapsillosis	+	++	+	+	+
C. pseudotropicalis	+	+++	++	+	+
C. steatolytica	+	++	++	++	++
C. tropicalis	+	++	++	+	+

N.B: (+) = active; (++) = moderate activity; (+++) = strong activity

Table IX. Quantitative determination of proteolytic enzyme (in µg/mL) of the selected *Candida* species at different pH values

pH values Candida species	Skimmed milk			casein		
	3.5	6.5	8.5	3.5	6.5	8.5
Candida albicans	420	190	305	390	235	315
C. guilliermondii	280	150	145	200	105	135
C. krusei	295	155	170	350	95	150
C. parapsillosis	290	130	110	295	130	95
C. pseudotropicalis	315	265	195	295	170	105
C. steatolytica	405	170	295	370	210	270
C. tropicalis	250	75	50	230	115	55

Table X. Antifungal sensitivity test against the selected *Candida* species

Candida species	Antifungal agent							
	Cy.	AB	NY	FC	EC	KT	MC	FZ
Candida albicans	-	+	+	+	-	+	+	+
C. guilliermondii	-	-	+	-	-	-	-	-
C. krusei	-	-	+	-	-	-	-	-
C. parapsillosis	-	-	+	-	-	-	-	-
C. pseudotropicalis	-	-	+	-	-	-	-	-
C. steatolytica	-	-	+	-	-	-	-	-
C. tropicalis	-	-	+	-	-	-	-	-

CY: Cycoheximide AB: Amphotericine B NY: Nystatin; FC: 5-Flurocytosine; EC: Econazole; KT: Ketoconazole; MC: Miconazole; FZ: Fluconazole; (+) = sensitive; (-) = resistant

DISCUSSION

The study of indoor yeast isolates specially of general sites e.g. Opera house (O) and Police prison rooms(P) is now of great importance because people spend some of their time inside theaters and some of them may be at risk, while many of the people inside Police prison rooms are immunocompromised and susceptible to fungal infection. The endiginous airborne yeasts should be studied intensively to elucidate their significance to public health. Research on seasonal periodicities of indoor yeasts will provide doctors with information needed for controlling airborne fungal spore population and will help to point the important allergic culprits in the indoor environment.

In our study, 860 cfu of indoor yeast species were counted. Similar results were obtained by the study of Cvetnic and Pepeljnjak (2001) that counted about 890 yeast colonies and suggested that the spores of these indoor fungi are known to be allergenic. This is also agree with the study of Su *et al.* (2001) who suggested that yeast was among the predominant genera of indoor airborne fungi. Also, the study of Ismail *et al.* (2000) reported that yeasts were among the most prevalent fungi from both indoor and outdoor microhabitats. Kuehn *et al.* (1995) suggested that yeast species was among the most common taxa identified in indoor environment. Duchaine *et al.* (2000) isolated various molds and yeast from eastern Canadian saw mils. Marchisio and Airaudi (2001) stated that yeasts were among the dominant indoor fungal aerosol of Italy. Bobichon *et al.* (1993) found that the floors of indoor swimming pools are contaminated by yeasts, dermatophytes and other saprophytic species.

In comparison of cultured yeast propagules among different sites of isolation, the results indicated that a high yield in terms of cfu were more consistently with the indoor Police prison rooms where 604 cfu was reported, while in Opera house it was (256 cfu). This result is agree with that of Pasanen *et al.* (1992) who reported that yeasts were more common and significantly higher in damp residences and old rural houses than other residences. Petroya *et al.* (2000) reported that *Cryptococcus albidus* and *Debaromyces hansenii* dominated in both abundance and frequency in indoor soil borne apartments in Moscow. These results somewhat similar to our observation in which *Cryptococcus albidus* was dominant in Opera indoor air in the four seasons and *Debaromyces hansenii* recovered from both Opera house and Police prison rooms.

The species composition of yeast isolates includes 25 species belonging to 10 genera. These are *Candida, Cryptococcus, Debaromyces, Endomyces. Geotrichum, Kloechera, Rhodotorula, Saccharomyces, Torulopsis* and *Trichosporon.* Our study showed that *C. famata* was among the yeast species with high count and this results was agree with results of Zhou *et al.* (2000) who reported that *C.famata* was among the indoor commonly found fungal species. *Cryptococcus albidus* was among the yeast species isolated in our study. The same genus have also been reported by Picco and Rodolfi (2000) who isolated *Cryptococcus neoformans* from outdoors and stated that the mycoflora of an indoor environment can depend both on fungal spora coming from outside and the capacity of fungi to colonize the different sublayers found indoor.

Saccharomyces cervisiae was among the indoor yeast species isolated with higher count in Police prison rooms (14 cfu) than Opera house (9 cfu) this may be due to individual crowding and bad ventilation in Police prison

Table XI. List of opportunistic yeast species isolated from Opera house (O) and Police prison rooms (P) in the four seasons

No	Yeast species	(O)				(P)			
		Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
1	*Candida albicans*	-	+	-	-	+	+	-	-
2	*C. catenulata*	+	+	+	+	+	-	-	-
3	*C. guilliermondii*	+	+	-	-	+	+	-	-
4	*C. krusei*	+	+	-	-	-	-	+	+
5	*C. type strain*	-	-	-	-	-	-	-	+
6	*C. parapsillosis*	-	+	-	-	+	-	-	-
7	*C. Pseudotropicalis*		-	-	-	-	-	-	+
8	*C. tropicalis*	+	+	+	+	+	+	+	+
9	*Cryptococcus albidus*	+	+	+	+	-	-	-	+
10	*Debaromyces hansenii*	+	+	-	-	+	+	-	-
11	*Kloechera apiculata*	+	+	-	+	+	-	-	-
12	*Rhodotorula glutinis*	-	-	+	-	-	-	-	-
13	*R. rubra*	-	-	-	-	-	+	-	-
14	*Torulopsis glabrata*	-	+	-	-	-	-	+	-
15	*Trichosporon beigelii*	-	+	-	+	-	-	-	-
16	*T. cutaneum*	-	+	-	+	+	+	+	+
17	*T. fermentans*	-	-	-	-	-	-	+	-

Table XII. Opportunistic yeast species isolated from Opera house and Police prison rooms currently known to cause human mycotic diseases

Yeast species	Associated diseases
Candida guilliermondii and C. tropicalis	Candidiases, otomycosis, mycotic keratitis, cutaneous mycosis, allergy and vaginitis
Cryptococcus albidus	Cryptococcosis (pulmonary).
Trichosporon beigelii and T. cutaneum	Pulmonary mycosis, mycotic keratitis and dermatophytosis
Rhodotorula glutinis	Pulmonary mycosis and mycotic keratitis .
Debaromyces hansenii	Bronchial allergy.

rooms. This result can agree with the observation of Rossi *et al.* (1995) who determined *Saccharomyces cerevisiae* in the indoor environment of a glass factory and suggested that the increase in mutagenicity of air samples collected near the machinery is due to the thermal decomposition of oils.

The genera of *Candida* and *Cryptococcus* were among the yeast genera isolated from indoor air of both Opera house and Police prison rooms. The count in cfu of *Candida* species in Police prison rooms is much higher than that of Opera house, this may be due to bad ventilation and individual crowding in Police prison rooms, on the other hand, count in cfu of *Cryptococcus albidus* is much higher in Opera house than Police prison rooms this may due to the air-conditioning systems distributed throughout the Opera house. A very similar categorization of indoor yeast isolates have also been reached by Cosentino *et al.* (1990) who recovered among the indoor fungi the genera *Candida* and *Cryptococcus* and reported that they potentially responsible for respiratory diseases. Their results support the idea that an air conditioning system can play a significant role in enhancing allergic and non-allergic diseases.

Rhodotorula glutinis was among the indoor yeast species recovered in our study this is supported by the study of Ripatti *et al.* (1990) who suggested that *Rhodotorula glutinis* was among the environmental microbes and attention should be focused on ventilation in stables.

Milton *et al.* (2001) reported that (1 fwdarw)–Beta–D–glucans such as that from *Saccharomyces cereviciae* have been recognized as a potential causative agent responsible for bio-aerosol-induced respiratory systems observed in both indoor and occupational environments. Raty *et al.* (1995) detected one antibacterial, one antifungal and three genotoxic strains among 12 fungal strains tested. Su *et al.* (2001) reported that seasonal effects seem to be a critical factor for the concentration and distribution of domestic endotoxin in study homes. Young *et al.* (2003) reported that 1 fwdarw 3-Beta-glucans derived from the inner cell wall of yeast and fungi and commonly found in indoor air dust samples have been implicated in organic dust toxic syndrome.

Seasonal variation showed that from Opera house, *Candida catenulata, C. tropicalis* and *Cryptococcus albidus* were recorded as a common isolates of the four seasons. But from Police prison rooms, *Candida tropicalis* and *Trichosporon cutaneum* were the most common isolates in the four seasons. However, the highest yield was obtained in autumn (546 cuf) and least in spring (59 cfu). Similar results were obtained by Mishra and Dwivedi (1982) who reported that *Fusarium*, *Curvularia* and *Cladosporium* were dominate all over the year, while yeast and penicillia were dominate in rainy season and *Alternaria* in summer.

The genera of yeast flora recorded in our study and reported as opportunistic pathogens (Mc Ginnis, 1980; Rippon, 1982) were *Candida, Cryptococcus, Debaromyces, Kloechera, Rhodotorula, Torulopsis* and *Trichosporon. Candida guilliermondii, C. tropicalis* and *C.krusei* are species of *Candida* that were incriminated as etiologic agents in the different types of Candidiases (mucocutaneous vaginitis, bronchial and pulmonary, paronchia and onychomycosis) as reported by Aronson and Soltani (1976).

Cryptococcus albidus was reported by Krumbolz (1972) to cause pulmonary cryptococcosis.

Chew *et al.* (1999) reported that *Candida albicans* was among the common indoor allergens and play a role in the pathogenisis of allergic respiratory diseases. Singh and Kumar (2002) suggested that *Candida aibieans* was among the important indoor allergens. Ruotsalainen *et al.* (1995) suggested that *Candida* spp. was among the indoor air fungi that may activate leukocytes to produce oxidative stress perhaps associated with harmful effects in exposed individuals. Norback *et al.* (2000) reported that indoor yeast and air pollutants in the classrooms air may influence nasal mucosa. Singh *et al.* (2000) reported that *Candida* triggers candidiases may be classified as cutaneous, mucosal, orpharyngal, oesophagal or deeply invasive of gastrointestinal tract, respiratory tract or urinogenital system of these, candidaemia-a vaginal infection is of great health concern because of increasing incidence in hospitals.

The genus *Rhodotorula* is common airborne contaminants, but it was reported in several acute infections of skin (Kramer & Koch, 1963), lung and urine (Ahern & Jannach, 1966) and eye infection (Seagal *et al*., 1975). Also Pore and Chen (1976) reported meningitis cases caused by *Rhodotorula.* The genus Trichosporon represented by *T.beigelii, T. cutaneum* and *T. fermentans* are common in soil, air, sputum and body surface. *T. beigelii* causes the well known infection of the hair shaft, the white piedra (Patterson *et. al.*, 1962). While *T. cutaneum* was reported as uncommon systemic pathogen in some rare infections (Madhavan *et al*., 1976).

Demonstration and identification of indoor yeasts serve various purposes. In general it gives an idea of which yeasts the people are exposed. This study gives useful information to allergists and other clinical practitioners in the study and treatment of fungal diseases. It will continue to prepare antigens to use in serological studies as well as in order to determine the significance of such diseases in Cairo and offer better means of diagnosis to the clinicians.

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