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Dynamics of human respiratory system mycoflora

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The study aimed at determing the prevalence of individual species of fungi in the respiratory systems of women and men, analysis of the dynamics of the fungi in individual sections of the respiratory system as concerns their quantity and identification of phenology of the isolated fungi coupled with an attempt at identifying their possible preferences for appearing during specific seasons of the year.

During 10 years of studies (1989–1998). 29 species of fungi belonging: *Candida, Geotrichum, Saccharomyces, Saccharomycopsis, Schizosaccharomyces, Torulopsis, Trichosporon* and *Aspergillus* were isolated from the ontocenoses of the respiratory systems of patients at the Independent Public Center for Pulmonology and Oncology in Olsztyn. *Candida albicans* was a clearly dominating fungus. Individual species appeared individually, in twos or threes in a single patient; they were isolated more frequently in the spring and autumn, less frequently during the winter and summer. The largest number of fungi species were isolated from sputum (29 species), bronchoscopic material (23 species) and pharyngeal swabs (15 species). *Saccharomycopsis capsularis* and *Trichosporon beigelii* should be treated as new for the respiratory system. Biodiversity of fungi, their numbers and continous fluctuations in frequency indicate that the respiratory system ontocenose offers the optimum conditions for growth and development of the majority of the majority of yeasts – like fungi.

Key words: yeasts – like fungi, respiratory system, biodiversity, prevalence.

INTRODUCTION

Pathogenic fungi can be found in the biosphere in different climatic zones. Most of the species causing mycosis in Central Europe are cosmopolitan (Kowszyk-Gindifer and Sobiczewski 1986) euryecological forms (Dynowska and Ejdys 1999). They usually colonize biotopes rich in saccharides and other organic compounds. They are isolated from the air, soil, fresh and marine waters, plankton, algae, fish, various plant organs (mainly fruit and seeds), gum and sap. Their habitat may be skin and its products (nails, hair), internal organs and whole systems, of humans and other vertebrates. In vertebrates pathogenic fungi are most often isolated in the case of a disease, endo- or exomycosis or immunological changes in the organism (D y n o w - s k a 1995).

Civilization development is accompanied by a growing number of factors conducive to the extension of the mycosis spectrum. This results from uncontrolled application of antibacterial antibiotics and environmental quality deterioration. Pathogenic fungi easily adapt to changing conditions and colonize new ecological niches.

In spite of great taxonomic diversity of potentially pathogenic fungi occurring in the respiratory system, the largest group are yeast-like fungi, discussed in this paper.

Modern classification and identification of potentially pathogenic fungi must be based on criteria corresponding with their biodiversity, species variation and flexibility connected with adaptability. The physiological response of fungi, reflecting their various sensitivity to the composition of the substratum and ontocenosis, is the most important microbiological criterion in the diagnostics of this group of organisms (D y n o w s k a 1995).

Fungal infections are usually recognized on the basis of clinical observations and laboratory investigations (R i c h a r d s o n and W a r n o c k 1995). The selection of research methods depends on the site of infection, symptoms and the clinical picture. A negative result does not exclude the possibility of a fungal infection.

The studies on respiratory system mycoses, conducted so far in Poland, were of a medical character, presenting concrete cases of mycosis (K r a k ó - w k a et al. 1990) or its relationships with other diseases, especially in predisposed patients (B a t u r a - G a b r y e l 1999; B a t u r a - G a b - r y e l et al. 1994; B r y l i ń s k a and Z a g ó r e c k a 1971; B r y l i ń s k a et al. 1976; B u d a k et al. 1995; C o p p o l a et al. 1993; G o r i et al. 1995; N a l e p a 1995). The following papers discuss the respiratory system only C h o d k o w s k a (1969), K u r n a t o w s k i (1980, 1992), B r y l i ń s - k a (1971, 1976), J e ż y n a (1975) and W e y m a n - R z u c i d ł o (1973, 1974). Numerous, but brief mentions of the subject can be found in some medical mycology textbooks (A l k i e w i c z 1966; P r o c h a c k i 1975; B a r a n 1998).

Lack of ecological surveys based on medical examinations that would investigate the respiratory system ontocenosis like a biocenosis made author follow the dynamics of the respiratory system mycoflora during 10 years.

Thanks to close co-operation between the Department of Mycology, University of Warmia and Mazury, and the Independent Public Center for Pulmonology and Oncology in Olsztyn author has access to diversified and very interesting clinical material coming from patients with respiratory and neoplastic diseases, living in the Warmia and Mazury Province. Dynowska has carried out investigations into the taxonomy, biology and ecology of pathogenic fungi in this region since 1986 (1990, 1991 – 1992, 1993a, 1993b, 1993e, 1995). She analyzed clinical material, looked for the same species of fungi in the external environment (D y n o w s k a 1993d; D y n o w s k a 1995), examined the growth rate of some fungi and some physiological processes (D y n o w s k a and B i e d u n k i e w i c z 1998; D y n o w s k a and G i e ł w a n o w s k a 1991 – 1992). New species appeared during the studies (D y n o w s k a 1996; D y n o w s k a and B i e d u n k i e w i c z 1999), other changed the frequency and range of occurrence (D y n o w s k a 1993c).

The majority of pathogenic fungi analyzed by D y n o w s k a (1995) come from the respiratory system, but this ontocenosis had not been presented within the space of many years. That is why this paper discusses some changes in the respiratory system mycoflora, taking into consideration the biodiversity and phenology of dominant fungi.

MATERIAL AND METHODS

In the period from January 1, 1989 to December 31, 1998 30 627 isolates coming from the respiratory system of patients treated at the Independent Public Center for Pulmonology and Oncology in Olsztyn were subjected to mycological analyses. The experimental material were swabs taken from the oral cavity, tongue, nose, pharynx and larynx, sputum – a mixture of secretions from the bronchi, upper airways (trachea, larynx), nasopharyngeal cavity, oral mucosa and salivary glands, and bronchoscopic material (BAL).

The cultures were incubated for 48 hours at a temperature of 37°C on the liquid Sabouraud medium. After that time the growth of fungi was determined according to the scale: poor, moderate, abundant and very abundant. Samples characterized by abundant and very abundant growth were inoculated into the solid (slants) and liquid (broth) Sabouraud medium. After another 48 hours of incubation at a temperature of 37°C, the following macroscopic traits of the colonies were examined: color, shape, consistency, area, margins, the way of growing into the medium. Cultures showing poor or moderate growth were disregarded during a detailed analysis of the isolates. In order to obtain pure non-bacterial strains, several passages were carried out, into a pure medium and a medium with antibiotics. Then the samples were cultured on the Nickerson agar. Microcultures were incubated for 48 – 72 hours at a temperature of 37°C. After that time the growth of fungi was studied under a microscope, evaluating the morphology of mycelia and their elements. Special attention was paid to the shape, formation and location of blastospores and chlamydospores.

The biochemical properties of fungi were determined using API tests (bioMérieux): API 20C and API 20C AUX. Gram-stained preparations were made from each kind of the material collected. Pictures were taken of macrocultures, microcultures and the above preparations. The fungi were identified according to the papers Barnett, P a y n e and Y a r r o w (1990), Lodder and Kreger – van Rij (1967), Kreger – van Rij (1984), Rieth (1983) and Kurnatowska (1995).

A statistical analysis was made using the computer program Statistica(r). It included a homogeneity test χ^2, an analysis of variance and a cluster analysis.

RESULTS

Quantitative and qualitative analysis of the material collected

In the period from January 1, 1989 to December 31, 1998 30 627 isolates were analyzed at the Bacteriological Laboratory, Independent Public Center for Pulmonology and Oncology in Olsztyn. The presence of fungi was confirmed in 10 031 (32.75%) cases (Table 1).

Table 1

Positive results obtained from clinical material coming from particular segments of the respiratory system vs. the total number of analyses (1989–1998)

Year	Total number of isolates	Positive results obtained from the sputum and pharyngeal swabs	Positive results obtained from the bronchoscopic material
1989	3105	712	145
1990	3359	730	185
1991	2773	721	231
1992	2569	865	267
1993	2703	831	261
1994	2657	620	248
1995	2890	426	267
1996	3218	694	348
1997	3371	834	296
1998	3982	1069	278
Suma	**30627**	**7505** (≈ 24,5%)	**2526** (≈ 8,25%)

Only samples abounding in fungi (4 076–40.63%) were subjected to further, detailed analyses. They were of primary importance in diagnostic procedures and therapeutic management. The highest number of isolates (80.42%) was obtained from the sputum; the fungi were less frequent in bronchoscopic material (13.71%) and pharyngeal swabs (5.28%). In the other kinds of material their share was minimal – 0.59%. Generally they were more often isolated from women – 2 359 than from men – 1 717. In a few cases (swabs from the oral cavity) the presence of fungi was observed only in women or men (swabs from the tongue, drain and tracheostomy tube). As regards the bronchoscopic material, fungi were isolated over three times more frequently from men than from women.

29 fungal species were isolated altogether. 8 taxa were not assigned to any species due to their mutually exclusive or "overlapping" morphological and biochemical properties (Table 2).

Table 2
Fungi isolated from the respiratory system ontocenosis (1989 – 1998)

No.	Species	Number of isolates
1.	*Aspergillus flavus* Link (1809)	1
2.	*Aspergillus fumigatus* Fresenius (1850)	1
3.	***Candida albicans*** **(Robin) Berkhout (1923)**	**2068**
4.	*Candida glabrata* Meyer et Yarrow (1978)	14
5.	***Candida guilliermondii*** **Langeron et Guerra (1935)**	**398**
6.	*Candida intermedia* Langeron et Guerra (1938)	43
7.	*Candida krusei* Berkhout (1923)	36
8.	*Candida lipolytica* Diddens et Lodder (1942)	9
9.	***Candida parapsilosis*** **Langeron et Talice (1959)**	**199**
10.	*Candida pseudotropicalis* Basgal (1931)	25
11.	***Candida tropicalis*** **(Castellani) Berkhout (1923)**	**116**
12.	*Candida utilis* Lodder et Kreger-van Rij (1952)	26
13.	*Geotrichum candidum* Link (1809)	2
14.	*Pichia farinosa* (Lindner) Hansen (1904)	2
15.	*Pichia membranaefaciens* Hansen (1904)	2
16.	*Saccharomyces cerevisiae* Meyen et Hansen (1883)	7
17.	***Saccharomycopsis capsularis*** **Schivnning (1903)**	**129**
18.	*Schizosaccharomyces versatilis* Wickerham et Duprat (1909)	2
19.	*Trichosporon beigelii* Vuillemin (1902)	69
20.	*Trichosporon capitatum* Diddens et Lodder (1942)	11
21.	*Trichosporon pullulans* Diddens et Lodder (1942)	3
22.	*Aspergillus* sp.	5
23.	***Candida*** **sp.**	**101**
24.	*Geotrichum* sp.	2
25.	*Pichia* sp.	15
26.	*Saccharomyces* sp.	24
27.	*Saccharomycopsis* sp.	55
28.	*Torulopsis* sp.	6
29.	***Trichosporon*** **sp.**	**196**

The main component of the mycocenoses examined were yeast-like fungi. Ten species from the genus *Candida* were isolated from the experimental material: *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. intermedia*, *C. krusei*, *C. lipolytica*, *C. parapsilosis*, *C. pseudotropicalis*, *C. tropicalis* and *C. utilis*. The genus *Trichosporon* was represented by three species: *T. beigelii*, *T. capitatum* i *T. pullulans*. Other yeast-like fungi were: *Geotrichum candidum* and *Saccharomycopsis capsularis*, and proper yeast included *Saccharomyces cerevisiae*, *Schizosaccharomyces versatilis* and *Torulopsis* sp. Two *Aspergillus* species were also isolated: *A. flavus* i *A. fumigatus*.

All of the fungal species presented in Table 2 were found in the sputum, 23 in the bronchoscopic material and 15 – in pharyngeal swabs. *Candida albicans*, *C. guilliermondii*, *Saccharomycopsis capsularis* and *Trichosporon beigelii* dominated in all kinds of the experimental material. Single isolates were obtained from the pleural cavity fluid, swabs from the oral cavity, tongue, nose, larynx, drain and tracheostomy tube. No statistically significant relationships were found between the share of fungi in particular kinds of the clinical material analyzed ($p > 0.05$).

An analysis of variance made to determine the variation of the fungi isolated in particular research years, taking into account their phenology, was statistically significant (Table 3). Most of them were isolated in autumn (1 333 cases) and spring (1 202), fewer in winter (979), the fewest in summer (562). The correlation between their occurrence and the season was similar in men and women ($p > 0.01$).

Table 3

Number of fungi isolated in particular seasons (1989–1998)

Year \ Season	Spring	Summer	Autumn	Winter
1989	104	23	131	55
1990	91	31	99	121
1991	121	72	132	84
1992	146	36	165	149
1993	143	56	166	121
1994	110	41	121	85
1995	124	44	119	70
1996	160	95	123	98
1997	105	58	109	105
1998	98	106	168	91
Suma	1202	562	1333	979

$\chi^2 = 165.09$, $p < 0.01$

A cluster analysis was made to determine the degree of similarity between particular fungal species. It was the highest in the case of *Candida albicans* and fungi belonging to three subgroups: *Saccharomycopsis capsularis*, *Candida tropicalis* and *Candida* sp., Trichosporon sp. and *Candida parapsilosis* and *C. guilliermondii*. These are the most dangerous potential pathogens, characterized by the most frequent occurrence. No clear similarity was found between the other species (Fig. 1).

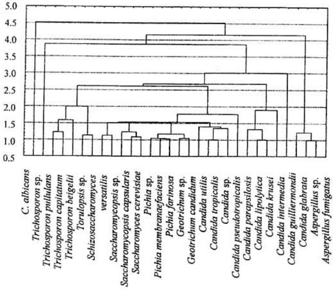

Fig. 1. Cluster analysis of species isolated individually

Characteristics of selected fungi, isolated most often from the respiratory system

The species isolated most often from the respiratory system is *Candida albicans* (57.91%); less common were: *Candida guilliermondii* (11.15%), *Candida parapsilosis* (5.52%), *Trichosporon* sp. – which had the majority of *Trichosporon beigelii* features (5.49%), *Saccharomycopsis capsularis* (3.61%) and *Candida tropicalis* (3.25%). The other fungal species constituted 13.27% of all isolates.

A detailed analysis concerned the most common species, which are of the greatest etiological importance as regards respiratory diseases. These are: *Candida albicans*, *C. guilliermondii* and *C. krusei* from the genus *Candida*, *Trichosporon beigelii* from the genus *Trichosporon*, *Saccharomycopsis capsularis* from the genus *Saccharomycopsis*.

The other species did not play an important role in the general picture of the ontocenosis studied.

Candida albicans – species isolated from 2 068 samples (57.9%), most often occurred alone, in 31 cases was accompanied by other fungi: *Saccharomycopsis capsularis*, *Candida tropicalis* and *Candida* sp., *Trichosporon* sp. and *Candida parapsilosis* and *C. guilliermondii*, with genus *Trichosporon: Trichosporon beigelii*, with genus *Saccharomycopsis: Saccharomycopsis capsularis* (Plate 2 A, B, C).

On the Sabouraud medium, after 24 hours, *C. albicans* forms cream-colored colonies, with smooth margins, slightly protruding above the agar surface, not growing into the medium, smooth and shiny. These colonies become wrinkled while aging (ca. two weeks) and give off a smell of yeast. In the Sabouraud broth they deposit sediment.

In microcultures on the Nickerson agar vegetative cells are spherical or cylindrical, and pseudofimbriae are surrounded with spherical blastospores. Older pseudofimbriae form lateral and terminal chlamydospores (Plate 1A, B, C). The number of *Candida albicans* changed inconsiderably only during ten years. This species was most frequently isolated in 1992, 1993 and 1996, and least frequently – in 1989 (Fig. 2).

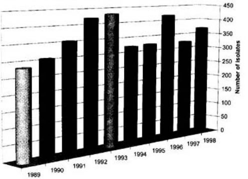

Fig. 2. Occurrence of *Candida albicans* in the respiratory system (1989–1998)

A gradual increase in the frequency of occurrence of *Candida albicans* was observed in both men and women until 1993. In 1994 a decrease in its number was noted, followed by an increase in 1996. The results obtained in the last two research years and in 1990 and 1991 are similar (Fig. 2). *Candida albicans* was

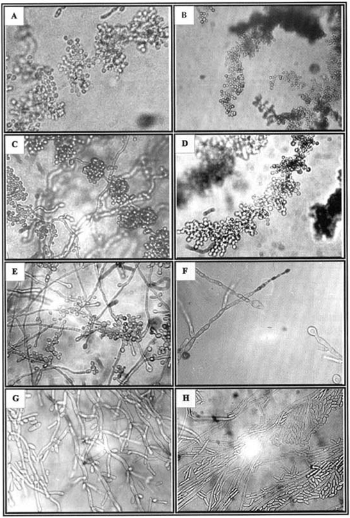

Plate 1. Microculture on the Nickerson agar (present individually): A – *Candida albicans* (pseudomycelium with blastospores); B – *Candida albicans* (chlamydospores); C – *Candida albicans* (typical scheme); D – *Candida guilliermondii* – characteristic blastospores scheme; E – *Saccharomycopsis capsularis*; F – *Saccharomycopsis capsularis* (chlamydospores); G – *Candida krusei*; H – *Trichosporon beigelii*

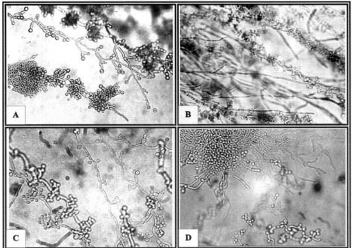

Plate 2. Microculture on the Nickerson agar (present in twos): A – *Candida albicans* + *Saccharomycopsis capsularis* (chlamydospores); B – *Candida albicans* + *Trichosporon beigelii*; C – *Candida albicans* + *Candida tropicalis*; D – *Trichosporon beigelii* + *Candida tropicalis*

more often isolated from the sputum (27.57% of cases) than from the bronchoscopic material (12.9%) (Table 4).

Tabela 4
Number of selected fungal species in particular kinds of material (1989–1998)

Material	*Candida albicans*	*Candida guilliermondii*	*Candida krusei*	*Saccharomycopsis capsularis*	*Trichosporon beigelii*
sputum	1652	330	25	105	46
bronchoscopic material	303	37	10	23	16
throat	100	24	1	1	5
oral cavity	6	–	–	–	–
larynx	1	–	–	–	–
tracheostomy tube	1	–	–	–	–
language	1	–	–	–	–
nose	1	1	–	–	–
fluid from the pleural cavity	1	–	–	–	1
drain	–	–	–	–	1
Total	2066	392	36	129	69

An analysis of the occurrence of *Candida albicans* shows that more isolates are obtained in spring and autumn than in winter and summer. Regardless of the season, a rapid decrease in the number of isolates was observed in 1994, and an increase in 1996 (Fig. 3).

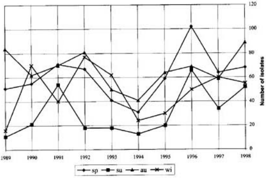

Fig. 3. Occurrence of *Candida albicans* in particular seasons (1989–1998)

Candida guilliermondii. The second most frequent species was Candida guilliermondii, isolated from 398 samples (11.15%). Together with other species it formed 16 different combinations. In most cases it was accompanied by *Candida albicans* i *Trichosporon* sp. sometimes by *Candida intermedia*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. tropicalis*, *C. utilis*, *Geotrichum candidum*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomycopsis* sp., *Trichosporon beigelii*, *Aspergillus* sp. + *Saccharomycopsis capsularis*, *Candida albicans* + *Candida intermedia*, *Candida parapsilosis* + *Torulopsis* sp. (Plate 1D).

On the Sabouraud agar it formed white colonies (turning yellow with culture aging), with smooth margins, not growing into the medium, shiny and smooth, giving off a smell of yeast.

On the Nickerson agar it forms cylindrical or egg-shaped vegetative cells, oval blastospores and branching pseudofimbriae (Plate 1D).

Since 1989 the number of *Candida guilliermondii* grew successively until 1994, when a rapid drop in its occurrence took place – the number of isolates decreased by a half. In the next three years of studies a further drop in its number was noted (Fig. 4).

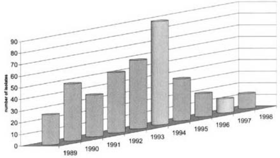

Fig. 4. Occurrence of *Candida guilliermondii* in the respiratory system (1989 – 1998)

Candida guilliermondii dominated in the sputum (330 isolates). It was seldom isolated from the bronchoscopic material (37 isolates) and pharyngeal swabs (24 isolates) (Table 4).

The most *Candida guilliermondii* isolates were obtained from the sputum in 1994. In consecutive years their number decreased considerably; a slight increase was noted in the last research year (Fig. 4). The frequency of *Candida guilliermondii* occurrence was similar in spring, autumn and winter, reaching a peak in 1992. In the other years it remained at a similar level (Fig. 5).

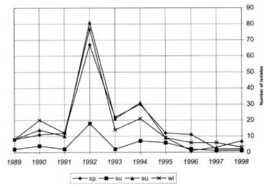

Fig. 5. Occurrence of *Candida guilliermondii* in particular seasons (1989 – 1998)

Candida krusei – is a species characterized by extremely high number fluctuations. Although the number of isolates is very small, compared with the other species described, its dynamics deserves an in-depth analysis, especially that for several years it was not present in the respiratory system. *Candida krusei* was noted in 36 cases only. 23 isolates were obtained in the first four years of studies, whereas 13 as late as in 1998. No isolates were obtained in the years 1993 – 1997 (Fig. 6). Together with the other species it formed 10 combinations: *Candida albicans, C. guilliermondii, C. parapsilosis, C. pseudotropicalis, Saccharomycopsis capsularis, Trichosporon beigelii, Trichosporon* sp., *Candida albicans* + *Candida utilis* i *Candida albicans* + *Trichosporon beigelii.*

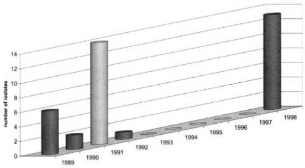

Fig. 6. Occurrence of *Candida krusei* in the respiratory system (1989 – 1998)

On the Sabouraud agar it produces white and cream-gray, mat colonies with rugged margins, looking like "frost on a window pane". The colonies protrude slightly above the agar surface and give off a yeast-like smell. On the Sabouraud broth it deposits sediment and forms a blanket, often causing turbidity of the whole medium.

In microcultures on the Nickerson medium it forms cylindrical, spindle-shaped, elongated or egg-shaped vegetative cells. Blastospores are elongated and form pseudofimbriae with side branches (Plate 1G).

Candida krusei was most often isolated from the sputum (25 isolates), less frequently from the bronchoscopic material (10 isolates). Only once a positive result was obtained in the case of a pharyngeal swab (Table 4). The most *Candida krusei* isolates were collected in autumn and summer. The number of isolates obtained in winter remained at the same, low level (Fig. 7).

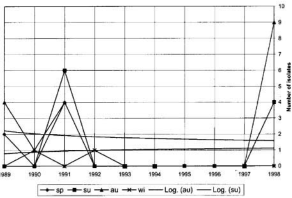

Fig. 7. Occurrence of *Candida krusei* in particular seasons (1989 – 1998)

Saccharomycopsis capsularis – *Saccharomycopsis capsularis* is a new species found in ontocenoses of the respiratory system. It s presence was confirmed in 129 cases. In 5 cases it was accompanied by other fungal species: *Candida albicans, C. glabrata, C. krusei, C. tropicalis, Trichosporon* sp.

On the Sabouraud agar *Saccharomycopsis capsularis* forms a cream-white mycelium, with a smooth, slightly protruding surface and smooth margins, rarely growing into the medium.

In microcultures on the Nickerson agar it forms oval or lemon-shaped vegetative cells and blastospores. On the mycelium clearly visible septa are surrounded with blastospores. In some strains fimbriae are wrinkled, and blastospores change their shape depending on the location on fimbriae. Cells similar to chlamydospores are formed at many places (Plate E, F).

Saccharomycopsis capsularis was noted for the first time in the ontocenosis examined in the fourth year of investigations. Within the space of ten years its number was increasing gradually from 1992 to 1996. The highest number of isolates was obtained in 1996. A lower frequency of this species occurrence was observed since 1997 (Fig. 8).

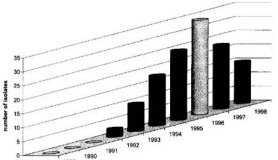

Fig. 8. Occurrence of *Saccharomycopsis capsularis* in the respiratory system (1989 – 1998)

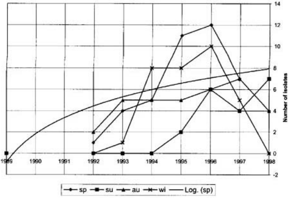

Fig. 9. Occurrence of *Saccharomycopsis capsularis* in particular seasons (1989 – 1998)

This species dominated in the sputum (81.4% cases), and was much less common in the bronchoscopic material (17.8% isolates). Only one strain was isolated from a pharyngeal swab (Table 4). The most isolates were obtained in spring and winter, fewer in autumn and summer (Fig. 9).

Trichosporon beigelii – is a species, which until 1992 was not noted in the respiratory systems of inhabitants of the Warmia and Mazury Province. The total number of its isolates (69) constitutes 1.7% of the data analyzed (it should be stressed that *Trichosporon* sp. had the most features of *Trichosporon beigelii*). It was also isolated in combinations with *Candida albicans, C. glabrata, C. guilliermondii, C. krusei, C. tropicalis, Rhodotorula* sp., *Saccharomycopsis* sp., *Candida albicans* + *Candida krusei.*

On the Sabouraud medium it has the form of fluffy, cream-colored colonies, protruding in the center, with rugged margins. On the Sabouraud broth it deposits sediment, produces a coarse-grained blanket and causes turbidity of the whole medium.

On microcultures on the Nickerson agar it forms egg-shaped, spherical or cylidrical vegetative cells and oval blastospores. The mycelium consists of elongated pseudofimbriae or fimbriae, assuming "rocket-shaped" forms and artrospores (Plate 1H).

Trichosporon beigelii appeared as late as in the fourth year of observations, and was present over the whole experimental period, although the frequency of its occurrence varied. The highest number of its isolates was noted in 1997, and the lowest – in 1994. In the other years it remained at a stable, low level (Fig. 10).

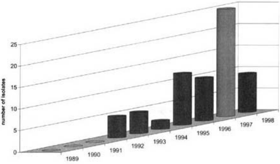

Fig. 10. Occurrence of *Trichosporon beigelii* in the respiratory system (1989 – 1998)

The most isolates were obtained from the sputum (66.7% cases), fewer from the bronchoscopic material (23.2% isolates). Sporadically *Trichosporon beigelii* was isolated from pharyngeal swabs (7.3%), the pleural cavity fluid and drain swabs – 1.4% of cases (Table 4). The highest number of isolates was collected in spring and autumn, the lowest – in summer and winter. As regards particular seasons (except for winter), the highest frequency of its occurrence was observed in 1997. In 1994 no isolates were obtained in summer and spring, whereas in 1992 and 1993 – in winter (Fig. 11).

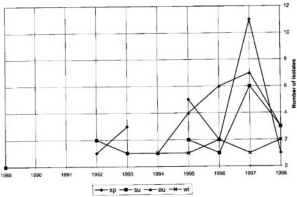

Fig. 11. Occurrence of *Trichosporon beigelii* in particular seasons (1989 – 1998)

DISCUSSION

A constant increase in the number of fungal infections and their widening spectrum (A g a b i a n et al. 1994; D y n o w s k a 1995; R i c h a r d s o n and W a r n o c k 1995), especially in predisposed people (Batura-Gabryel et al. 1994; B a t u r a - G a b r y e l 1999; D e P a u w 1999; M a r t i n o et al. 1993; Z a g ó r e c k a 1971), indicate the need to monitor the ontocenoses of the systems colonized most frequently by pathogenic fungi. One of them is the respiratory system, exposed to fungal infections, which may lead to systemic infections. The presence of fungi in the oral cavity or sputum does not necessarily indicate pathogenesis, but their presence in the bronchial secretion (B i e d u n k i e w i c z 1999) constitutes a serious threat to the patient's health and life. According to D y n o w s k a (1995), wide variation, flexibility and adaptability of the etiological agents discussed, resulting from their perfectly developed enzymatic system, as well as their fast rate of growth, allow them to colonize various ecological niches, including the human organism. It is more and more difficult to differentiate between particular ecological groups of fungi potentially pathogenic to humans. As a consequence, the same species appear simultaneously in several new ontocenoses (D y n o w s k a 1993c).

In order to examine the biocenotic interactions between organisms forming organic ontocenoses, and to analyze the properties of particular etiological agents (enzymatic activity, drug-sensitiveness) we must first of all know the species composition, which enables preliminary determination of the ontocenosis character. That is why basic research should not be neglected, as it allows to determine the direction of changes caused by macro- and micro-environments. Evidence supporting this thesis may be the results of the present studies, which show how dynamic a mycocenosis of one system can be, and how fast it changes when there appear fungi typical of other ontocenoses. An example is here *Trichosporon beigelii* – a keratinphilous species, which until quite recently was characteristic of skin (D y n o w s k a 1996; G u e m o et al. 1994). Its natural habitat is usually the soil. It was also noted in various types of water bodies (D y n o w s k a 1995). In own research *T. beigelii* was isolated in 1992, and the frequency of its occurrence was increasing every year. Another species which until recently was not isolated from the respiratory system in Poland is *Saccharomycopsis capsularis*. B a r n e t t, P a y n e and Y a r r o w (1990) report that its habitat may be the soil and grains of pollen of some tropical plants. In Poland this fungus is hardly known and rarely placed on the list of potential pathogens. In the Warmia and Mazury Province it was isolated for the first time by D y n o w s k a (1995) in 1992, from lakes, astatic water bodies and the respiratory system. This species, similarly to *T. beigelii*, appeared in 1992 and showed similar frequency of occurrence.

Observations of hospitalized patients in own studies show that numerous environmental factors are conducive to the development of pathogenic fungi. These are, among others, a relatively high temperature, high humidity and limited oxygen availability. Lack of proper ventilation in hospital rooms results in quick growth of fungi. As regards hospital infections, it is alarming that a great number of isolates was obtained during routine bacteriological examinations. The disease agents found in hospitals are subject to constant fluctuations. Some of them get there by the agency of fungus carriers whose carrier-state does not have to be connected with disease symptoms. Another group of people transporting new strains to hospitals are patients with asymptomatic mycoses, which may reveal during hospitalization or remain latent if not diagnosed properly. Own observations show that this is a relatively common phenomenon. In the last ten years a rapid increase in the number of hospital infections was noted. They were usually caused by *Candida* spp. This concerns hospitals in many European countries and the USA, especially intensive care units (G a r c m a - M a r t o s et al. 1996; S o b e l and V a z q u e z 1990; U t z 1987). Own results correspond closely with those obtained by the above-mentioned authors, concerning the most frequent species, such as: *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida lipolytica* and *Trichosporon beigelii*.

The biological material analyzed in the present studies was collected from patients suffering from various respiratory diseases. Due to the specific character of the hospital where the research was conducted, there was high percentage of patients with neoplastic diseases and with suspected tuberculosis. The occurrence of tuberculosis symptoms, when the presence of mycobacteria was not confirmed, does not necessarily suggest disease development. In spite of that, patients are in most cases treated with antibiotics, which considerably weakens their natural protective barriers. Because fungi, including yeast-like ones, often imitate tuberculosis symptoms, it is possible that many serious respiratory diseases are life-threatening, unrecognized mycoses. Similar conclusions were formulated by Varthalitis and Meunier (1995) on the basis of postmortem examinations. These observations indicate again the importance of detailed mycological analyses in patients from risk groups, especially those hospitalized many times and whose diseases had not been fully diagnosed. This is of primary significance taking into account cytotoxic carcinogenic activity of mycotoxins and enzymes produced by fungi (Batura-Gabryel et al. 1994). It seems that chronic and untreated mycoses may lead to cancerous lesions.

Increasing anthropopressure and environmental contamination, as well as more and more "aggressive" therapies (antibacterial antibiotics with a wider and wider spectrum, cytostatics, corticosteroids, immunosuppressive drugs), result in distinct differences in the biodiversity and range of occurrence of potentially pathogenic fungi. It follows that these fungi should be permanently monitored and their drug-sensitiveness should be determined as soon as the disease process starts. This is especially important since more and more *Candida albicans, Candida krusei* and *Candida glabrata* strains show natural drug-resistance to basic antimycotic drugs – ketoconazole and fluconazole (Baran 1998).

Fungi present in the human organism not always cause a disease, but in the case of reduced immunity they may lead to severe infections (Pappagianis 1967; Utz 1987). The mucosa of the upper airways is the first natural barrier stopping microorganisms. Fungi, which pass through it, can get to the lower airways: trachea, bronchi and lungs. It follows that it is very important that doctors looking after patients, especially those with chronic (e.g. neoplastic) diseases, differentiate between fungal colonization and an invasive infection (Varthalitis and Meunier 1995).

Yeast-like fungi (Y) appear at early stages of infections. Their blastospores are resistant to phagocytes. The mycelial form (M) penetrates into cells or tissues easier than blastospores, and appears later. Practice and own investigations indicate that both form can be observed in direct preparations. Therefore, it is assumed that both of them play an important role in causing an infection (Macura 1993; Macura-Biegun and Macura 1997). However, form M is more pathogenic than form Y. This is of special significance when fimbriae are observed in direct preparations obtained from the bronchoscopic material and sputum. During the transition from the yeast-like to mycelial form the composition of the cell wall changes, enabling formation of the so called "germ tubes" (Macura 1993).

Also vitamin (e.g. vitamin B) deficiency, application of corticosteroids and cytostatics, and chronic, devastating diseases (e.g. tuberculosis and neoplastic diseases) have a significant effect on epithelium susceptibility to fungus development (Fedorowicz 1999; Rawińska-Zakrzewska et al. 1994; Zagórecka 1971; Jeżyna 1975; Zaremba and Borowski 1997; Batura-Gabryel 1999). Fungal infections are often the main cause of diseases or even death of hospitalized patients. In many cases fungi are found in almost all kinds of biological material taken from the infection focus. In own studies fungi were much more frequently isolated from patients with neoplastic diseases than tuberculosis. In the case of such patients it is very important to conduct an *in vitro* test of adherence to human epithelial cells. This enables the determination of mutual interactions between a potential pathogen and the host organism (Macura 1994). During colonization and infection many dynamic changes take place in the relation fungus-host, evoking defense reactions on the side of the fungus or host (Batura-Gabryel 1999).

Passing through successive biological and mechanical barriers, fungi colonize various organs. The sources of infection are often skin and mucous membranes, colonized asymptomatically. Studies aimed at showing the biodiversity of fungi colonizing particular ontocenoses are conducted at many mycological centers in Poland. In the course of research carried out in the years 1977–1991 at the Mycological Laboratory, Hospital of the Ministry of Internal Affairs in Poznań fungi were isolated from the oral cavity, sputum and pharynx. The majority (61%) of strains belonged to *Candida albicans* (Maleszka et al. 1993). At present this is the most common pathogen. This was confirmed also by own investigations, in which this species constituted 57.9%. Similar data were gathered in the region of Gdańsk (Nowicki and Sadowska 1993) and in Warsaw (Krajewska et al. 1998). Own results correspond with those obtained at the Institute of Stomatology, Medical University in Łódź (Kurnatowska 1981), where *Candida albicans, C. tropicalis, C. krusei, C. parapsilosis, C. pseudotropicalis, C. utilis* and *Geotrichum candidum* were isolated from the oral cavity. The majority of fungi isolated in own studies also represented the genus *Candida*, and 2/3 of them belonged to the species *Candida albicans*.

An analysis of the material collected shows that except for *Candida albicans* the occurrence frequency of the other species (*Candida tropicalis, C. pseudotropicalis, C. krusei, C. guilliermondii, C. parapsilosis*) in the respiratory system decreased. Parallel research conducted by Dynowska (1990) indicate similar composition and fluctuations of mycoflora in the same ontocenoses. This tendency is also confirmed by the results obtained by Kurnatowski et al. (1996), concerning mycological characteristics of the homogenate of palatine tonsils removed due to chronic inflammatory changes. In his studies on the species composition of mycoflora in healthy people, Majewski (1973) isolated from the oral cavity first of all *Candida albicans* (31) and

C. pseudotropicalis (17), rarely *C. parapsilosis, C. humicola, C. mesenterica and C. tropicalis*. Similar composition of the oral cavity ontocenosis was reported by Kurnatowska (1981), Omulecki and Krykowska-Koniarek (1997).

Mixed infections with several fungal species are very common. This concerns mainly the genus Candida (*Candida tropicalis, C. pseudotropicalis, C. krusei, C. guilliermondii, C. parapsilosis*). In the present investigations combinations of the genus *Candida* with other genera, e.g. *Trichosporon* or *Saccharomycopsis*, were also noted frequently. This is probably connected with increasing biodiversity of pathogenic fungi in organic ontocenoses, and their growing expansiveness. High species diversity is accompanied by regular occurrence of particular species, which is consistent with a general biological tendency concerning the functioning of multi-species associations.

A decreasing number of some species (even periodically dominant) is often accompanied by an increase in the number of other, frequently considered harmless pollutants. This concerns first of all people with reduced immunity (Biedunkiewicz 1999; Dynowska 1996; Dynowska and Biedunkiewicz 1999). An example may be *Candida krusei*, which was isolated in the first four research years, and then disappeared for consecutive five. It was noted again as late as in 1998.

In 1994 a rapid decrease in the number of all fungal species isolated in the studies took place, followed by a gradual increase. Probably at the beginning it was caused by the introduction and common use of a wide assortment of mycostatics of a new generation, and later on – by gradual acquisition of resistance by fungi, often being a consequence of incorrect therapy and improper selection of antimycotic drugs (Baran 1998). The application of the right dose of drugs ensures their high mycostatic or mycodical level in the blood (Siwińska-Gołębiowska 1990).

The development of mycoses is stimulated first of all by disturbances in the natural microorganism equilibrium, caused by excessive, thoughtless and uncontrolled application of antibacterial antibiotics and other chemotherapeutic agents. Similar conclusions were formulated by Varthalitis and Meunier (1995). They found out that disturbances in endogenous flora not only stimulate the growth of fungi but are also conducive to their colonization of internal organs, including penetration of mycelium elements into the blood, which transports them all over the organism. They usually stay in damaged tissues, characterized by lower oxidation-reduction potential (Richardson and Warnock 1995).

A constant increase in the occurrence of secondary fungal invasions has its roots in the introduction of antibacterial and antimycotic antibiotics, cytostatics, corticosteroids and immunosuppressive drugs, combined with resistance acquisition by strains of potentially pathogenic fungi (Borowski 1973; Jeżyna 1975; Kowszyk-Gindifer and Sobiczewski 1986; Rieth 1983; Seelig 1966; Seeliger and Tintelnot 1990). This is proved by numerous reports about a growing incidence of blastomycoses (Alvarez et al. 1990; Bodey 1984; Dynowska 1990, 1993; Kurnatowska 1995) and data concerning resistance transmission not only among fungi, but also between fungi and bacteria. Long-term drug treatment increases the resistance of pathogenic fungi or leads to selection of resistant strains, not excluding accumulation of side effects (Rogowska-Szadkowska 1996; Ruhnke, Tennagen and Engelmann 1994). Pathogenic fungi are able to transfer, from one cell to another, genetic information determining antibiotic resistance. Apart from selective role of antibiotics, this is the main cause of the appearance of resistant strains in a hospital environment (Dzierżanowska 1990). Antigens of two different fungal species (e.g. *Cryptococcus neoformans* and *Trichosporon* sp.) present in the organism can induce cross reactions (Herbrecht 1993).

An obstacle to the effectiveness of antimycotic drugs is high enzymatic activity of fungi, connected with their adaptation to environmental conditions (Larski and Truszczyński 1997; Maleszka and Baran 2000). Another important reason for low effectiveness of mycostatics is polymorphism of fungi and the fact that in the organism they appear close to necrotic lesions and in poorly vascularized regions, where biochemical conditions are not conducive to drug diffusion (Maleszka and Baran 2000; Pawlik 1986). An additional problem are capsules made of material stimulating fungus growth (Rieth 1983). Antibacterial antibiotics may also constitute an additional source of energy for many fungi, which use their C and N (Jeżyna 1975). There is a philogenetic relation between fungi and bacteria, which is the cause of problems in antimycotic therapies. Antifungal drugs, which inhibit the synthesis of DNA and RNA or fungus protein, may have the same effect on human cells (Virella 2000).

To sum up – an ideal antimycotic drug should be characterized by high clinical effectiveness, confirmed by negative results of mycological examinations, low percentage of disease recurrence and a short treatment time, without side effects and interactions with other commonly applied medicines (Odom 1996).

Apart from tuberculosis and neoplastic diseases, also other pathological factors increase the predisposition to mycoses. These are: diabetes, chronic obturative pulmonary disease and insufficiency of the immune system (Bodey 1984; Jawetz et al. 1974; Nawrot 1997; Rieth 1983; Sobel and Vazquez 1990; Zaremba and Nowacki 1990; Coppola et al. 1993; Listemann and Meigel 1995). In order to minimize the risk of fungal infections, all patients considered predisposed to them should be preventively given antimycotic drugs before and during chemotherapy or antibiotic therapy (Varthalitis and Meunier 1995).

Potentially pathogenic fungi, as heterotrophic components of the biosphere (specialized reducers, decomposers), process and use organic compounds produced by autotrophic organisms (Dynowska 1996). They may be considered euryecological organisms, present in all climate zones, in habitats characterized by a wide range of temperatures, humidity, pressure, substratum acidity and a various oxygen content (D y n o w s k a 1995). They colonize biotopes rich in carbohydrates and other organic compounds. They are isolated from the air, fruit and seeds, cereal grains, insects, plankton, algae, crustaceans and fish, fresh and marine waters (A l - D o o r y 1968; D y n o w s k a 1993a, 1995; D y n o w s k a and B i e d u n k i e w i c z 1998; S k i n n e r and F l e t c h e r 1960; W e y m a n - R z u c i d ł o 1974). Mycelium elements or spores can travel between the aerosphere, hydrosphere, lithosphere and human organism, causing home and environmental infections (K u r n a t o w s k a 1973). Mycological analyses of water ecosystems in the Warmia and Mazury Province made by D y n o w s k a (1995) allowed to isolate 33 species of potentially pathogenic yeast and yeast-like fungi. During parallel studies on the clinical material obtained from patients from the same area, she isolated 16 fungal species, out of which as many as 14 were earlier found in water samples. Most of the fungi isolated by D y n o w s k a (1995) came from municipal sewage. These results suggest that there is a close correlation between degradation and contamination of water environments, and the occurrence of fungi in clinical material obtained from patients living in this region.

Fungi are constantly present in the biosphere and human organism. Therefore, composition changes observed in the mycoflora analyzed in the present investigations are connected not only with environmental sanitation, but also with the physical condition of patients, affected by their immunological state and widely understood antibiotic therapy (D y n o w s k a 1995, 1996).

Fungi are characterized by wide morphological and physiological variation, reflecting their adaptation to changing environmental conditions. This variation may be pheno- or genotypic (L a r s k i and T r u s z c z y ń s k i 1997).

Being saprothrophs, they more and more often colonize the human organism, becoming potential pathogens (R i c h a r d s o n and W a r n o c k 1995). One of them is *Saccharomycopsis capsularis*, representing the family *Cryptococcaceae* (B a r n e t t et al. 1990). Observations made in the Warmia and Mazury Province show that *Saccharomycopsis capsularis* found favorable conditions for growth and development in the respiratory system (D y n o w s k a 1995; B i e d u n k i e w i c z 1999). Although according to R i e t h (1983) *Saccharomycopsis* species do not play an important role in epidemiology, the results obtained by D y n o w s k a (1993 a, 1995) seem to contradict this opinion. She emphasizes a growing occurrence of the species discussed and its abundance. The studies presented in this paper confirm these observations. *Saccharomycopsis capsularis* isolated from patients may have an immunosuppressive effect (K u r n a t o w s k a 1995), and its metabolism products can be toxic or carcinogenic.

The existence of fungi in the external environment depends first of all on temperature and humidity, but their occurrence is also determined by the season, region and climate. A humid and warm climate, with heavy precipitation, as well as water bodies located nearby, are conducive to the development of hydrophilous fungi (B u s h 1989). In a moderate climate, such or similar conditions prevail in autumn and spring, i.e. the seasons when the greatest number of isolates was obtained. These transitory periods (spring and autumn) are accompanied by infections and intensive antibiotic application, resulting in reduced immunity and increased susceptibility to fungal invasions. Their seasonal incidence can be discussed from the perspective of fungal invasiveness and activation of mycelial forms. The available literature on the topic contains no detailed information on the occurrence of respiratory system infections caused by yeast-like fungi in particular seasons. An exception are mentions of an increased number of positive results (by ca. 15–25%) obtained from the oral cavity ontocenosis in summer (K u r n a t o w s k i 1986), which may be related with consumption of large amounts of fruit, constituting a perfect medium for fungi. Studies conducted in a tropical, warm and humid climate usually concern infections with dermatophytes (C h a n d e r and S h a r m a 1994; C h e b o t a r e v et al. 1987); other analyze the effect of air conditioners installed inside buildings, which change the air composition and contribute to the occurrence of fungal infections with *Aspergillus*, *Alternaria*, *Cladosporium* and *Penicillium*, sometimes also *Candida* (C o s e n t i n o et al. 1990). The interpretation of the results obtained, taking into account climatic or biocenotic aspects, is difficult, because all of the species described in this paper are very common in various parts of the world and in the biosphere. Similarly, it is not possible to enumerate the species showing affinity with the respiratory system, as they are also isolated from other ontocenoses.

The human organism is an ontohabitat colonized by various microorganism species. Healthy people have their own physiological flora (creating a specific microenvironment), which protects them against invasions of pathogenic flora. Commensal and symbiotic flora consists mainly of bacteria and fungi (B e r k o w et al. 1995). Thanks to a phenomenon known as tissue tropism, microorganisms colonize certain tissues only. Commensal flora restores quickly when disturbed, whereas temporary flora appears for hours or weeks only. The relationships between the host and microorganisms, and the biocenotic interactions between organisms living in organic ontocenoses, should be the subject of further ecological and epidemiological investigations.

During ten years of own studies, constant changes were observed in the fungal species colonizing the respiratory system. An exception was *Candida albicans*, which dominated over the whole experimental period. *Candida parapsilosis*, *Candida guilliermondii*, *Candida tropicalis* and *Candida krusei* could also be considered dominant species at the beginning of the research, but later on their share diminished considerably. *Saccharomycopsis capsularis* and *Trichosporon beigelii* may be treated as new species in the respiratory system ontocenosis, which seems to provide excellent conditions for their growth and development. Attention should also be paid to *Candida krusei*, which appeared, dominated and then disappeared for five years. It seems to be connected with changes in the interactions between particular species, constituting an indicator of widely understood parabiosis, and with maintaining the internal equilibrium of the organism, also at the immunological level. In such situations only dynamic systems are able to survive – stagnant ones are gradually dying. Dynamic equilibrium is a natural phenomenon. It leads to homeostasis not only at the level of cells and organisms, but the whole population, and is sometimes referred to as "living in harmony with nature and oneself". Traditional ecological studies ignore this aspect: humans are considered separate beings, and no attention is paid to the environment that surrounds them.

According to Spooner (1984), the basis of ecology is observation of both changes in the composition of multi-species ontocenoses in the host organism and external environment ecosystems (Kurnatowska 1997), and relationships between organisms and their surroundings.

The present paper not only shows a wide variety of fungi colonizing the respiratory system, but also emphasizes the need to monitor fungal species. This is of primary significance taking into consideration increasing drug-resistance of fungi and decreasing immunity of humans to fungal infections, directly correlated with the development of medicine and pharmacology (Baran 1998). The paper determines the directions of further research in this field, including an analysis of the enzymatic activity of the most expansive species, and a correlation between the presence of fungi in a given organism and the secretion of enzymes playing the most important role in the pathogenesis of mycoses.

CONCLUSION

1. During ten years of observations (1989–1998) 29 fungal species were isolated from the respiratory system ontocenosis of patients treated at the Independent Public Center for Pulmonology and Oncology in Olsztyn. The fungi represented the following genera: *Candida*, *Geotrichum*, *Saccharomyces*, *Saccharomycopsis*, *Schizosaccharomyces*, *Torulopsis*, *Trichosporon* and *Aspergillus*.
2. The species isolated most often were: *Candida albicans*, *C. guilliermondii*, *C. parapsilosis* i *C. tropicalis*, *Trichosporon beigelii* and *Saccharomycopsis capsularis*. The most dominant among them was *Candida albicans*. The frequency of occurrence of the other ones was subject to considerable fluctuations, resulting from their periodical activity affected directly by the condition of the whole organism.
3. In the last years of investigations a distinct decrease was noted in the incidence of *Candida guilliermondii*, *Saccharomycopsis capsularis* and

Trichosporon beigelii. The last two species were new for the respiratory system, so from the moment of their appearance in this ontocenosis they showed expansiveness and a high frequency, which decreased with time. This was probably a consequence of multi-directional impacts and a tendency towards the coexistence of all microorganisms within the ontocenosis.

4. The highest number of species – 29 was obtained from the sputum, 23 from the bronchoscopic material, and 15 from pharyngeal swabs. Taking into consideration all kinds of the clinical material analyzed, fungi were more often found in women (mainly *Candida albicans, C. guilliermondii, C. parapsilosis, Saccharomycopsis capsularis* and *Trichosporon beigelii) than in men (mainly Candida krusei* and *Trichosporon beigelii*). An exception was the bronchoscopic material – here fungi were isolated over three times more frequently from men than from women.
5. One, two or three fungal species were isolated from single patients. If there were three of them, their growth was always abundant or very abundant, with gemmating cells and, in some cases, pseudofimbriae with blastospores in direct preparations.
6. The most fungi were isolated in spring and autumn, fewer in winter and summer. However, no phenological correlation was noted between particular species and seasons. The differences in the frequency of their occurrence are probably connected with the degree of invasiveness, a periodical drop in the immunity of the organisms examined and conducive iatrogenic factors, different in particular seasons.
7. The biodiversity of fungi, their number and constant changes in the species composition of ontocenoses show that the microenvironment studied offers optimum conditions for the existence of all fungi, which can grow and develop at human body temperature.
8. The research results confirm that the occurrence spectrum of potentially pathogenic fungi becomes wider and wider. They also indicate the need to monitor them in order to find those characterized by the highest expansiveness, which may be estimated on the basis of their enzymatic activity and drug resistance.

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Dynamika mikoflory układu oddechowego człowieka

S t r e s z c z e n i e

Celem badań było określenie prewalencji poszczególnych gatunków grzybów występujących w drogach oddechowych kobiet i mężczyzn, przeanalizowanie dynamiki grzybów poszczególnych odcinków układu oddechowego pod względem ilościowym i jakościowym oraz określenie fenologii wyizolowanych grzybów z jednoczesną próbą określenia ewentualnych ich preferencji do występowania w konkretnych porach roku.

W ciągu 10 lat badań (1989—1998) z ontocenozy układu oddechowego pacjentów Samodzielnego Publicznego Zespołu Pulmonologii i Onkologii w Olsztynie wyizolowano 29 gatunków grzybów z rodzajów: *Candida, Geotrichum, Saccharomyces, Saccharomycopsis, Schizosaccharomyces, Torulopsis, Trichosporon* i *Aspergillus*. Zdecydowanym dominantem była *Candida albicans*. Poszczególne gatunki występowały pojedynczo, po dwa lub rzadziej po trzy u jednego pacjenta, częściej izolowano je wiosną i jesienią, rzadziej zimą i latem. Najwięcej grzybów uzyskano z plwociny (29 gat.), materiału bronchoskopowego (23 gat.) i wymazów z gardła (15 gat.). *Saccharomycopsis capsularis* i *Trichosporon beigelii* należy traktować jako nowe dla układu oddechowego. Bioróżnorodność grzybów, ich liczba i ciągłe fluktuacje we frekwencji dowodzą, że ontocenoza układu oddechowego stwarza optymalne warunki dla wzrostu i rozwoju większości grzybów drożdżopodobnych.