

Original papers

Yeasts isolated from frequently in-patients and out-patients

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ABSTRACT. The paper is a part of a general mycological monitoring study of nosocomial infections. Differences in the species composition and drug resistance of fungi isolated from in-patients and out-patients prompted an analysis of this topic. The studies were conducted with fungi from the collection of the Department of Mycology, University of Warmia and Mazury in Olsztyn, that were isolated from the oral cavity, sputum, bronchoscopic fluid, anus and skin from 100 in-patients and 100 out-patients. Laboratory analyses, including species categorization of fungi, were performed according to routine mycological diagnostics. Drug sensitivity to fluconazole and nystatin was tested with the disc diffusion method. In the group of in-patients, a wider taxonomic diversity of fungi (12 species) was found in comparison with the out-patients (7 species) and 31 cases of multifocal infections were recorded, while in the second group the number of the latter was only six. In all patients, *C. albicans* were predominant, constituting the largest proportion in focal infections in all patients and in multifocal infections in the in-patients. In the latter, over a half of the examined individuals were resistant to fluconazole (*C. glabrata* and *C. krusei* – 80%, *C. tropicalis* and *S. capsularis* – 60%, *C. guilliermondii* – 50%) and nystatin (*T. beigelii* – 80%, *C. krusei* and *C. tropicalis* – 50%). Substantially lower drug resistance of fungi was recorded in the out-patients. The hospital environment is an abundant reservoir of different fungal species with significantly greater expansiveness and aggressiveness compared to the environment outside a hospital.

Key words: yeasts, in-patients, out-patients, fluconazole, nystatin

Introduction

Yeasts are a polyphyletic and heterogeneous group of microorganisms that include anamorphic and heteromorphic Ascomycota and Basidiomycota of different taxa. According to Kurtzman et al. [1] the latest view, yeasts and yeast-like fungi are incorporated into this group; budding is their common feature. Many of them are important in medical mycology as the causative agents of endogenous and exogenous mycoses or they are enumerated as commensals of the oral mucosa, digestive tract, respiratory tract, vagina and less frequently, the skin [2,3].

The results of mycological analyses, carried out for 20 years, of biological material sampled from the respiratory and digestive tract of healthy and diseased individuals clearly indicate that a taxonomic and ecological spectrum of fungi in the organ ontocenoses is expanding [4,5]. Multifocal colonization is being reported with an increasing frequency and it poses a risk of fungal invasions being spread with the blood to different organs [4,6,7]. The number of iatrogenic factors is obviously rising and they are responsible for an increase in the expansiveness of fungi towards human tissues, with the human onthosphere being one of the natural reservoirs [8]. In particular, this concerns persons with a disrupted biological balance and impaired immune system who are colonized by fungi asymptomatically or reveal clinical symptoms [9–12]. Such symptoms are usually noted while performing bacteriological tests (fungal infections often mimic bacterial infections) upon a doctor's request for diagnostic and/or therapeutic purposes [8,10].

During monitoring studies carried out in cooperation with the Independent Public Complex of Tuberculosis and Lung Diseases in Olsztyn, it was found that in one-third of the in-patients, fungal infections occurred during hospitalization. In as many as 23.3% of the examined individuals, these infections were multifocal and caused by drug-resistant and selected strains found in their ontocenoses or in their surroundings [13].

It was decided to compare the positive results of mycological analyses for frequent in-patients and out-patients with a focus on drug-resistance in fungi to fluconazole and nystatin, i.e. mycostatics that are very often used not only for therapeutic purposes but also in a protective manner together with antibacterial treatments and chemotherapy.

Materials and Methods

The studies were carried out with fungi (200 isolates) from the collection of the Department of Mycology, University of Warmia and Mazury in Olsztyn, that were isolated from the oral cavity and sputum, bronchoscopic fluid, anus and skin (intedigital spaces in the feet) from 100 in-patients of the Independent Public Complex of Tuberculosis and Lung Diseases in Olsztyn and from 100 out-patients treated in different specialist clinics. Macrocultures (Sabouraud agar with chloramphenicol or gentamicin) and microcultures (Nickerson agar) as well as species categorization of fungi (GRASO chromogenic tests, zymograms, auxanograms), were performed in accordance with routine mycological diagnostics [1–3,14–16]. In both groups of patients, the species structure of fungi and their presence in one focus (focal infection, FI) and in multiple foci (multifocal infection, MFI) were compared. The drug sensitivity of the obtained isolates to fluconazole and nystatin was tested with the disc diffusion method.

Results

In the group of in-patients, a higher taxonomic diversity of fungi (12 species) was found in comparison with the out-patients (7 species) together with 31 (31%) cases of multifocal infections, while the number of the latter in the second group was only six (6%) (Table 1, Fig. 1). In all patients, *Candida albicans* was the predominant species, constituting as much as 62% in the out-patients and 45% in the in-patients (Table 1). It is emphasized that a relatively high proportion of *Candida tropicalis* and *Trichosporon beigelii* were found in focal infections in the out-patients (Table 1, Fig. 1). In addition, greater differences were reported in the number of isolates obtained from the individual onthocenoses and biological materials in comparison with the in-patients (Fig. 2A, B). In the latter, except for the skin, the numbers of isolates are comparable (Table 1, Fig. 2B). In the out-patients,

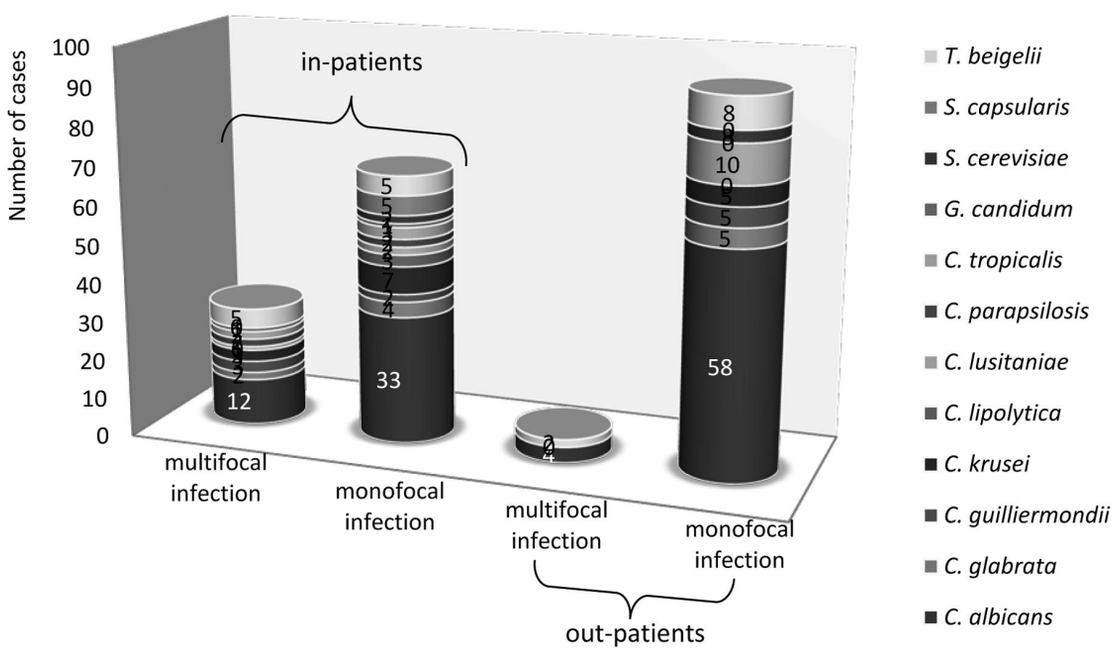

Fig. 1. Monofocal and multifocal infection of particular species of fungi in hospitalized patients and outpatients

Table 1. Comparison of the taxonomic spectrum and the number of isolates from biological materials in-patients and out-patients, including monofocal and multifocal infection

No.	Species of fungi	The biological material / number of cases = number of isolates															
		in-patients								out-patients							
		oral cavity	sputum	bronchoscopic fluid	skin	anus	monofocal infection	multifocal infection	sum	oral cavity	sputum	bronchoscopic fluid	skin	anus	monofocal infection	multifocal infection	sum
1	*C. albicans*	12	15	10	3	5	33	12	45	24	15	0	15	8	58	4	62
2	*C. glabrata*	2	2	0	0	2	4	2	6	2	3	0	0	0	5	0	5
3	*C. guilliermondii*	2	1	1	0	1	2	3	5	1	1	0	1	2	5	0	5
4	*C. krusei*	3	3	3	0	1	7	3	10	2	1	0	0	2	5	0	5
5	*C. lipolytica*	1	1	0	0	1	3	0	3	0	0	0	0	0	0	0	0
6	*C. lusitaniae*	1	0	0	0	2	2	1	3	0	0	0	0	0	0	0	0
7	*C. parapsilosis*	1	1	1	0	1	2	2	4	0	0	0	0	0	0	0	0
8	*C. tropicalis*	2	2	1	0	0	3	2	5	5	2	1	0	2	10	0	10
9	*G. candidum*	0	0	0	1	1	1	1	2	0	0	0	0	0	0	0	0
10	*S. cerevisiae*	5	0	0	1	0	2	0	2	3	0	0	0	0	3	0	3
11	*S. capsularis*	2	0	0	1	0	5	0	5	0	0	0	0	0	0	0	0
12	*T. beigelii*	1	1	0	4	4	5	5	10	1	1	0	4	4	8	2	10
13	**Total**	**32**	**26**	**16**	**10**	**18**	**69**	**31**	**100**	**38**	**23**	**1**	**20**	**18**	**94**	**6**	**100**

the highest number of isolates was sampled from the oral cavity, a comparable number from the sputum, skin and anus, and only one isolate from the bronchoscopic fluid (Table 1, Fig. 2A). In the case of one species, i.e. *Trichosporon beigelii*, a clear relation of co-occurrence in the skin and anus was reported in all patients (Table 1, Fig. 2A, B).

Over half of the isolates that were retrieved from the in-patients showed resistance to fluconazole (Fig. 3). It mainly applies to *Candida glabrata* and *C. krusei* (80%) and to a lesser extent to *C. tropicalis* and *Saccharomycopsis capsularis* (60%) and *C. guilliermondii* (50%). Lower, yet more diverse resistance was reported for nystatin (Fig. 4) in *T. beigelii* (80%), *S. capsularis* (60%) and C. *guilliermondii*, *C. krusei* and *C. tropicalis* (50%). Substantially lower resistance to both drugs was detected for isolates from the out-patients (Fig. 3, 4). In this group, the highest resistance to fluconazole and nystatin was found in *C. glabrata*, i.e. 50 and 30%, respectively (Fig. 3, 4).

Discussion

With the current state of knowledge in mycology and ecophysiology of fungi, it is obvious that fungi not only attack diseased people more often than healthy individuals [2,5,8,11], but the type of disease and its course and treatment are also important [10,17–20]. The conducted studies indicate that the place in which a patient stays plays a considerable role [21]. Despite continuous improvements in asepsis and antiseptics, the hospital environment still remains a specific reservoir of fungi from different taxa and bio-ecological groups [13,21–23]. A hospital is also a place in which all links of the epidemiological chain of mycoses are found: sources of infections, ways of spreading (much shorter than outside hospitals) and individuals with increased susceptibility. Susceptibility to fungal infections is determined by non-specific and specific resistance mechanisms which are exposed to more disturbing factors in a

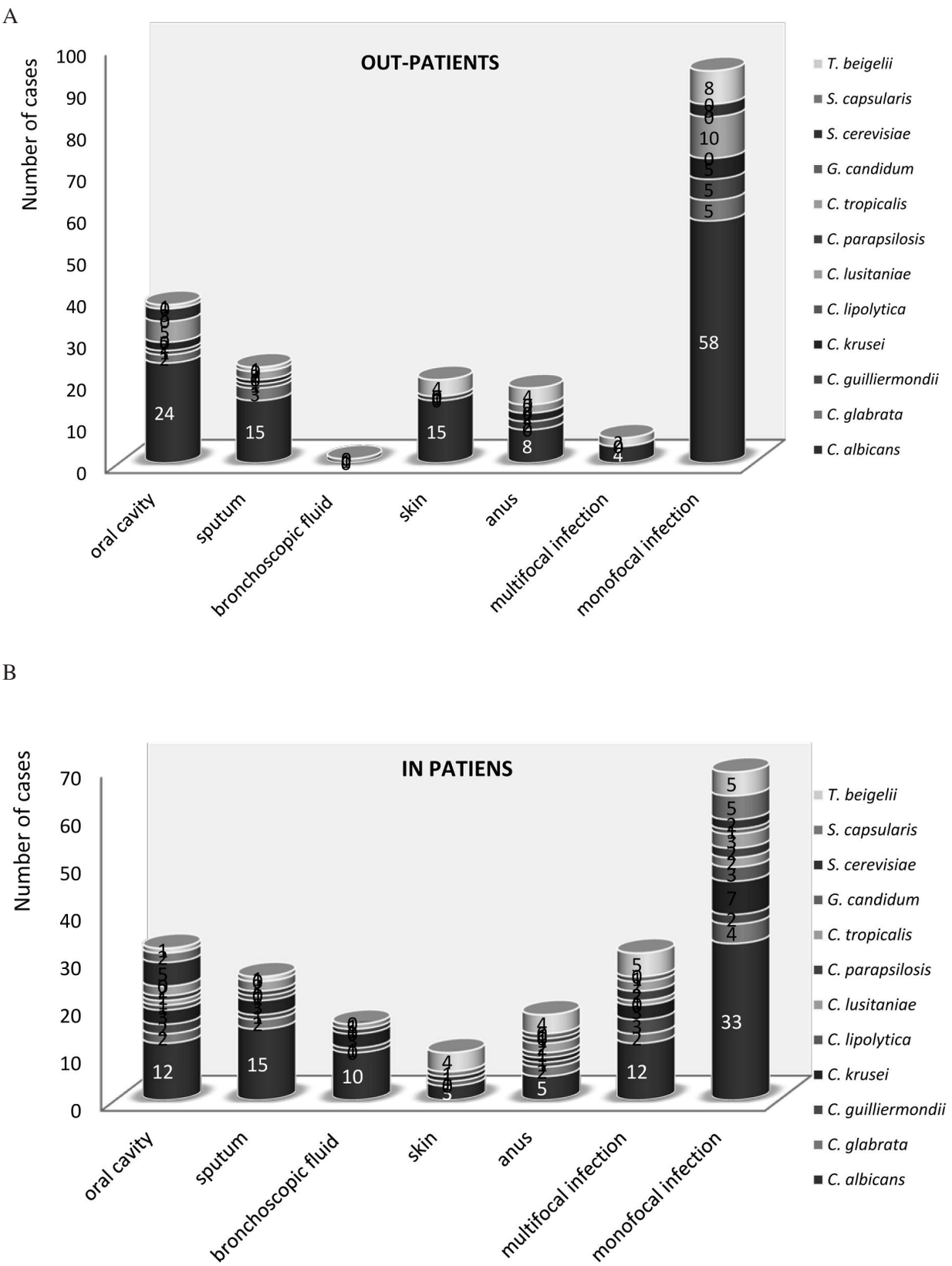

Fig. 2. Numerical differentiation of isolates obtained from particular ontocenoses and biological materials out-patients (A) and in-patients (B)

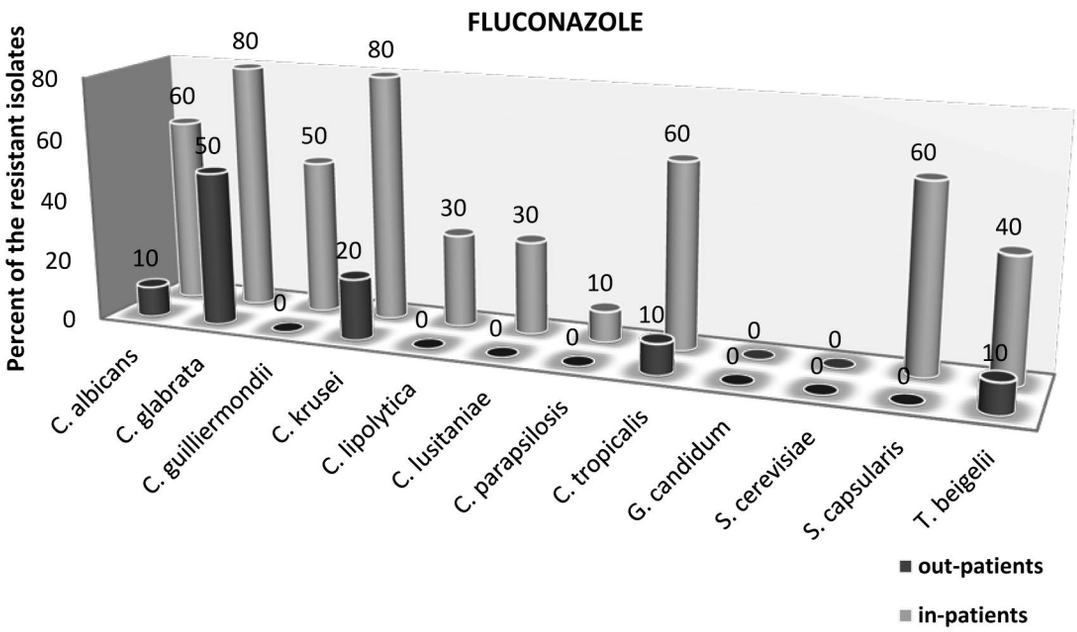

Fig. 3. Percent comparison of drug-susceptibility on fluconazole of fungi, isolated from biological materials out-patients and in-patients

hospital than outside it. Psychoimmune factors should not be overlooked as they are important for regaining biological balance and homeostasis. Since out-patients are usually treated at home in the usual human and microbial environment, recovery and restoration of the biological balance may be easier than in a hospital. Based on the obtained results, it is difficult to unambiguously state which of the isolated species colonized the examined patients during a primary disease and therapy and which had previously colonized them. In many people who are frequently diseased and treated with antibiotics,

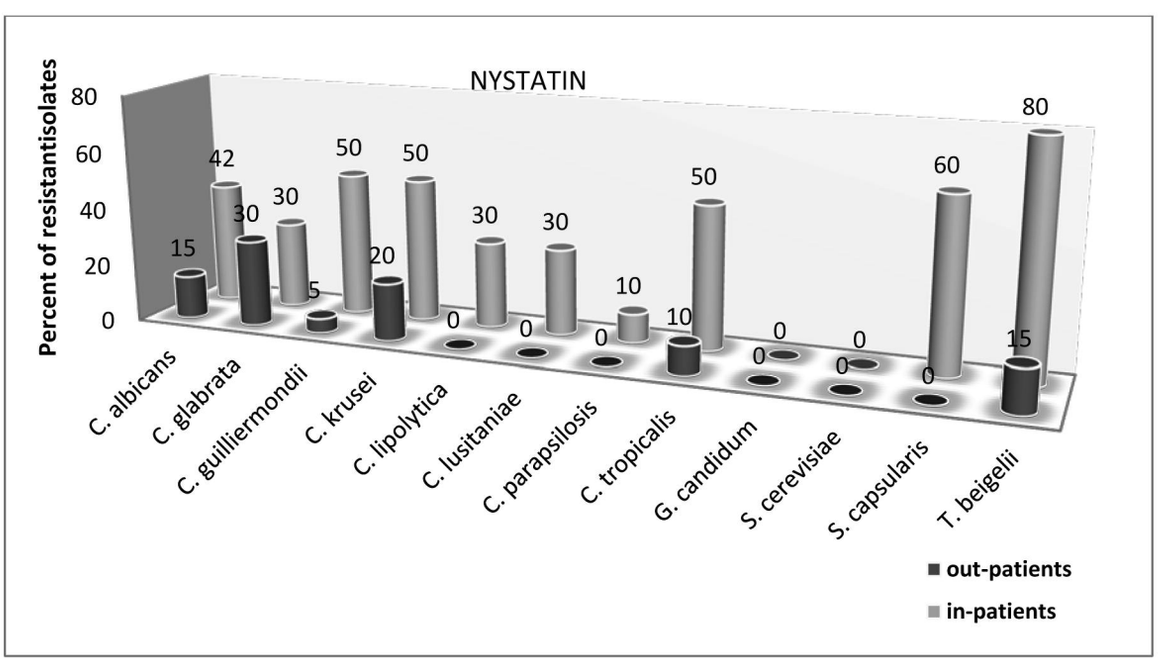

Fig. 4. Percent comparison of drug-susceptibility on nystatin of fungi, isolated from biological materials out-patients and in-patients

especially those with a wide spectrum of activity, some fungal species occur chronically [10,24,25] and accompany inflammatory conditions of different origins [4,8]. These include *Candida albicans* and *C. tropicalis*, which was clearly confirmed in the conducted studies. *Trichosporon beigelii* simultaneously colonized the skin and anal mucosa in all patients. Previous studies have indicated that this species is expansive and increasingly often attacks the organ ontocenoses, particularly the digestive tract [8] and respiratory tract [4,5,7,9]. Due to its limited living space, the hospital environment is a place where competitive phenomena are potentiated and very often lead to increased metabolic activity and aggressiveness of a variety of microorganisms, including fungi. Such competition may result in eliminating weaker species or strengthening them and enriching the biodiversity [26] in a given ontocenosis and environment. It probably explains why the percentage of multifocal infections is so high in hospitals and the persistently high biodiversity of fungi may indicate a specific allelopathy.

Following a similar pathway of working in relation to the results for the out-patients, it is supposed that an open living space and a greater ecological capacity should weaken competitiveness and ecological expansiveness as it is demonstrated with a low percentage of multifocal infections and relatively low drug resistance. However, much more frequent isolations of *C. albicans* from out-patients than in-patients indicate an affinity of this species to the human body and the potential of its colonization even at times of a transient physiological imbalance. Patients colonized by drug-resistant pathogens may be difficult to detect, as in a few of them clinical symptoms are present, yet they are a source of infection – they are sometimes called "a silent source of infection" [10,21].

An in-patient is exposed to nosocomial strains and those introduced by patients. Strains that are successively transported to a hospital by patients are faced with ecophysiological barriers formed by isolated and selected intra-hospital populations that are well-adapted, active, sometimes even expansive and very often resistant to mycostatics. Most probably, they transfer this feature to all fungi in their surroundings. The hospital environment is thus an important link in the transmission of fungi and in disseminating information on drug sensitivity [27–29].

Disturbing reports by Dean et al. [30] indicate that approximately 33% of in-patients undergoing a prophylactic antifungal treatment develop mycosis. This observation and the results of the present studies show the high risk of exposure of in-patient to fungal infections. Apart from the above-mentioned ecological reasoning, predisposing factors include changes in the endogenous microbiota, impairment of the immunity by a primary disease and applied treatment and the use of catheters or intravenous drug administration. Frequent use of the increasingly common antifungal prophylaxis without mycological analyses results in survival of resistant strains [4,5]. Destruction of the intestinal barrier, frequent immunosuppression and previous colonization of the mucosal membranes by fungi are the major risk factors for fungemia and deep systemic mycoses [5,8,31]. The use of antifungal drugs with a spectrum that does not cover a given fungus being the causative agent of a disease and repeated treatments with the same drug are the most common mistakes [32]. It also creates a space for resistant species to increase in number. The results clearly indicate that fluconazole is an overused drug [13,32,33]. Therefore, constant mycological, environmental and individual monitoring is necessary in a hospital; it should include all patients and be carried out in parallel with bacteriological monitoring [13,21]. It would then allow for identifying patients who are colonized by fungi and for targeting eventual treatments and prophylaxis, especially in mixed infections.

The vast majority of the data on medical mycology indicates that the growth in fungal infections is associated not only with predisposing factors of an autogenic and allogenic nature that are increasing in numbers, but mainly with the growing expansiveness of fungi in human surroundings as they circulate in the whole biosphere, including the ontosphere [5,6,25]. The occurrence of new species in the human ontosphere [8,34] also results from decreasing resistance to fungal infections and a progressively weaker opposition of the physiological microbiota to colonization [4,25] and, as a result, easier transformation of macroorganisms from eubacteriosis into dysbacteriosis. The hospital environment clearly has a stimulating impact on this process [35].

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