

Results of long-standing mycological analyses of biological materials originating from selected organ ontocenoses – yeast and yeast-like fungi[1]

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ABSTRACT. This paper reviews the results of an extensive monitoring study, spanning 20-years of observation from three medical centres in Olsztyn on the dynamics and species diversity of fungi most frequently colonizing the respiratory and digestive systems of humans. The experimental materials were swabs and specimens from the gastrointestinal tract, swabs from the oral cavity and pharynx, as well as sputum and bronchial fluid from the respiratory system. The biological material was subjected to routine mycological diagnostics, taxonomic determination and identification. In total, 41 species of yeast and yeast-like fungi were isolated, including 34 from the respiratory and 25 from the digestive system. In the last decade, a significant increase has been noticed in the counts of fungi, especially the gastrointestinal tract, reported from people. As many as 18 species were isolated from both systems – they were predominated by fungi of the genus *Candida* and their perfect forms. Worthy of notice are also frequent isolations of yeast (*Saccharomyces* spp.) and detection of an endemic species, *Paracoccidioides brasiliensis,* in the respiratory system, and of the sexual stages of *Rhodosporidium diobovatum* and *Rhodosporidium kratochvilovae* from the gastrointestinal tract.

Key words: respiratory system, digestive system, yeast, yeast-like fungi, comparative analysis

Introduction

Investigations conducted in the Department of Mycology, University of Warmia and Mazury in Olsztyn, in collaboration with other clinical centres, have focused for many years primarily on two organ systems in the human body, namely: the respiratory and digestive systems. Through their natural openings to the exterior, these systems are exposed to all environmental contaminants, hence they are relatively often colonized by opportunistic fungi, particularly yeasts and yeast-like fungi (e.g. *Candida*, *Geotrichum*, *Trichosporon*, *Rhodotorula*, and *Saccharomyces* species), that primarily inhabit the common section, i.e. the oral cavity, that leads to both organ systems [1–3]. This is a gateway to infections organisms and an ecological niche with the greatest microbiological biodiversity. Here the fate of multiple potentially-pathogenic microfungi is decided [4]. These fungi may colonize both these systems asymptomatically or their presence may be signaled by pathogenic lesions.

Although knowledge about potentially pathogenic fungi of humans has developed

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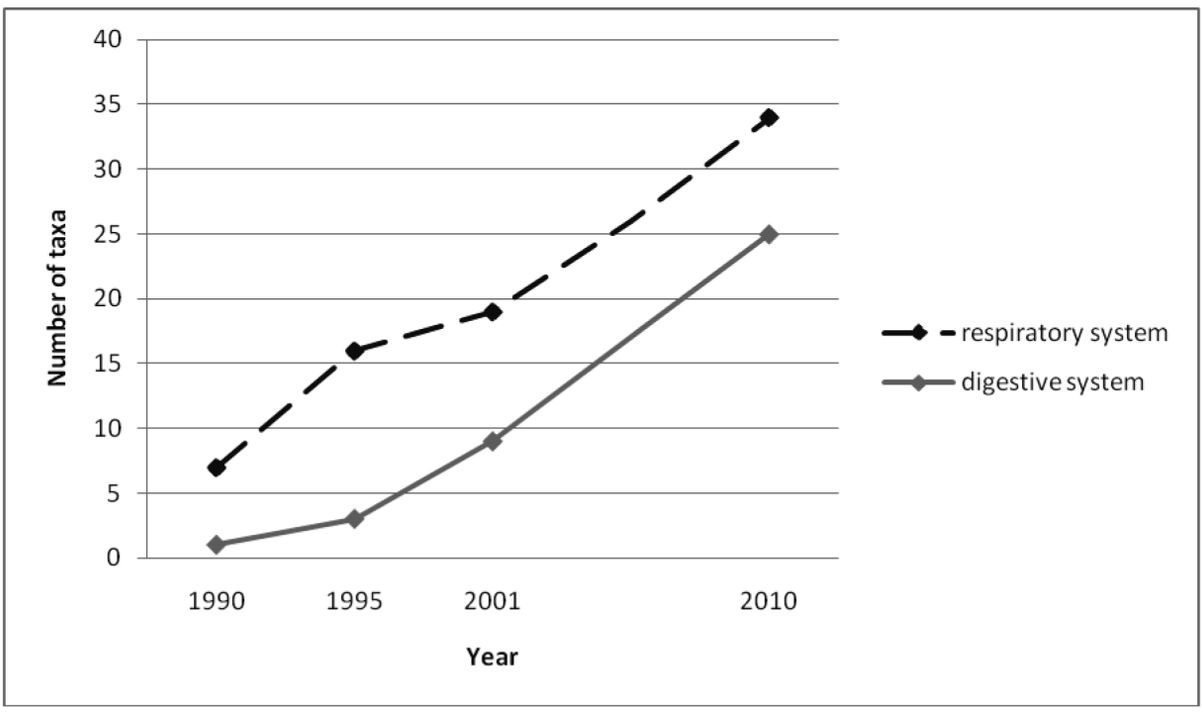

Fig. 1. Changes in the number of taxa of fungi isolated from humans during the last twenty years

significantly in recent years, there are still relatively few comparative works based on long-standing investigations focusing on the habitats presented in the respiratory and intestinal systems. In 1986, a program of monitoring studies was initiated in the Department of Mycology, University of Warmia and Mazury in Olsztyn, covering implementing commonly employed mycological analyses of biological samples from infected as well as normal subjects, including adults [5–7], adolescents [8] and children [9], as well as analyses of samples originating from their environments [10–12]. The objective of this monitoring study is to evaluate the taxonomic and ecophysiological spectrum of fungi capable of colonizing man's ontocenoses, to determine the rate of invasion of new ontocenoses by species that are already known or those that have not been described as yet, and to evaluate the phenomenon whereby some species displace others.

The results of mycological monitoring may serve as a background and starting point for in-depth clinical and environmental surveys and may determine priorities and directions of these surveys. In view of the above, this work is an attempt to summarize the most significant results of these fundamental studies conducted in the years: 1990–2000, 2001–2010.

Material and methods

Biological materials were collected by expert physicians, following standard procedures. They originated from different sections of the respiratory system (swabs, sputum, bronchoalveolar lavage –

Table 1. Number of taxa of fungi isolated in each year from respiratory and digestive system

Year	Respiratory system	Digestive system	References
1990	7	1	Dynowska [5]
1995	16	3	Dynowska [6,7]
2001	19	9	Biedunkiewicz [17] Dynowska and Biedunkiewicz [24] Dynowska et al. [29]
2010	34	25	Dynowska et al. [21] Dynowska et al. [4] Góralska [20] Dynowska et al.[23]

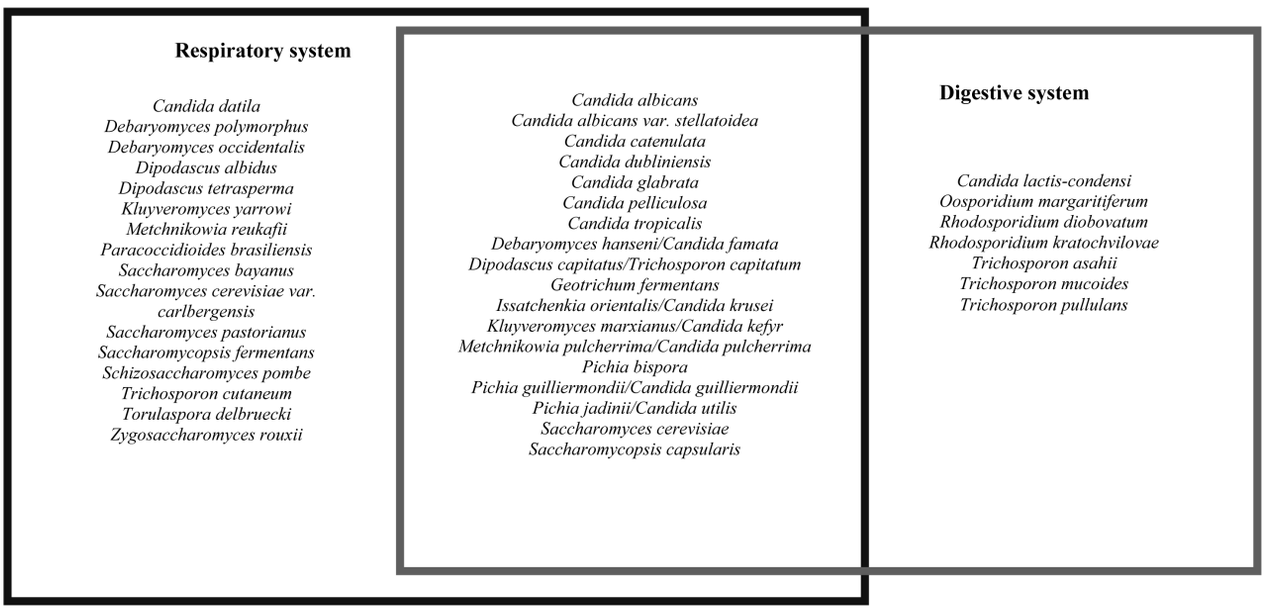

Fig. 2. Species of fungi isolated from respiratory and digestive system of man

BAL) and digestive system (swabs, specimens) of patients from three medical centres in Olsztyn, including: the Independent Public Hospital of Tuberculosis and Pulmonary Diseases in Olsztyn, the Clinical Ward of Oncological Surgery at the Hospital of the Ministry of Internal Affairs and Administration, and the Endoscopic Laboratory of the Municipal Hospital in Olsztyn. The most numerous group of patients were these with neoplasms, tuberculosis, and asthmatic lesions and with chronic inflammatory conditions. The other patients suffered from failures of various origin and earlier un-diagnosed ailments – in the latter case lesions with mycotic etiology were the most frequent.

From 100 to 140 isolates were collected each year, according to standard procedures employed in routine mycological diagnostics [2,13]. Macrocultures of fungi were incubated on Sabouraud's agar with the addition of chloramphenicol and gentamycin for 48–72 hours, at a temperature of 37°C. Having established fungal growth, the material was subinoculated onto liquid Sabouraud's medium for proliferation, and then passaged two or three times on solid medium (in order to eliminate bacteria). From the resultant isolates, microcultures were established on Nickerson's agar enriched with blood serum (1:1). After incubation at a temperature of 37°C for 72–144 h, the fungi were identified to species level using reference works by: Kurnatowska and Kurnatowski [2], De Hoog et al. [14], and the keys by: Kreger-van Rij [15] as well as Kurtzman and Fell [16]. The fungi were identified based on macroscopic, microscopic and biochemical traits (fermentation and assimilation capabilities of saccharides). Differentiation of species belonging to the genus *Candida* was also carried out with the use of Chromagar by GRASO.

On the basis of this 20-year investigation, a glucose-potato agar (PDA) is postulated as the best medium for routine diagnostics of clinical materials, being preferred by facultative saprophytes, an increasing number of which is being isolated from the ontosphere.

Results

The analyses have shown explicitly that the ontosphere of man is successively colonized by ever increasing numbers of taxa. This is evident from a comparative analysis of the results of significant references prepared based on cyclic reports and individual surveys. The years: 1990, 1995, 2001, and 2010 (Table 1), are worthy of special emphasis. Although in the first 10 years the increase in fungal counts was slow, the number of fungi observed in the second decade increased rapidly (Fig. 1).

Within 20 years, a total of 41 species of fungi were isolated from the investigated systems, including: 34 from the respiratory system and 25 from the digestive systems. Of these, 18 species

were isolated in both systems, whereas 16 taxa occurred exclusively in the respiratory system and 7 taxa exclusively in the digestive system (Fig. 2). The predominating fungi were these belonging to the phylum Ascomycota, class Endomycetes, order Saccharomycetales – as many as 26 species, whilst only one species – *Paracoccidioides brasiliensis* was identified as belonging to the order Onygenales. Mitosporic fungi (asexual forms until recently referred to as Deuteromycota) were represented by 8 species, including 7 that belonged to the genus *Candida*, and one to *Oosporidium margaritiferum*. Furthermore, 5 species (*Trichosporon asahii*, *Trichosporon cutaneum*, *Trichosporon mucoides*, *Rhodosporidium diobovatum* and *Rhodosporidium kratochvilovae*) were identified to belong to phylum Basidiomycota, class Urediniomycetes, order Sporidiales.

Discussion

Being opportunistic forms, yeast and yeast-like fungi constitute an asymptomatic component of the different sites of the ontosphere, but they usually colonize skin and its products as well as systems having direct contact with the external environment [2,4,17]. Parts of these fungi penetrate the body for nutrients and fluids, especially these sources containing carbohydrate-rich raw materials [18]. The risk posed by these fungi and the initiation of their pathogenic potential emerge usually when homeostasis is disrupted at the level of ontocenosis in which interactions of fungi with other microorganisms relatively often lead to the predominance of the former, especially in patients burdened with the primary disease [2,13,17,19].

The comparative analyses carried out in the current study demonstrate that both the respiratory and digestive systems constitute a favorable ecological niche for fungi, where they can grow and proliferate as well as adjust their properties to the biotic and abiotic characteristics of the microhabitat [20]. This fact is indicated by the isolation from the biological material the sexual stages, which in the case of fungi usually appear as a result of changes in habitat conditions [4,20]. Thus, these results corroborate the indistinct ecological borderline between various trophic groups of potentially-pathogenic fungi. An increasing number of these fungi have been reported to penetrate the respiratory and digestive systems [20–23]. In the case of yeast and yeast-like fungi, it is not possible to identify particular species as being typical of certain systems. In predisposed persons they are indicative of skin and organ blastomycosis, very often multi-focal ones [2,6,24]. Results referring to the last decade are alarming, including a 200% increase in the number of taxa isolated from the respiratory system and up to a 300% increase in the number of these isolated from the digestive tract, as compared to the previous decade. This should be a signal for introducing routine mycological analyses of all biological materials of persons predisposed to mycotic infections to the current procedures of bacteriological diagnostics.

Since nine more species were isolated from the respiratory system compared with the digestive system, this confirms the speculations of Góralska [20] that the former system provides better conditions for the survival and growth of fungi – greater stability of habitat conditions and greater access to oxygen. In the author's opinion, however, this does not exclude the fact that the digestive system remains the most frequent route of intraorganic transmission of fungi [4,25,26].

Worthy of special attention is the fact that Góralska [20] isolated 5 species that so far have not been noted in Poland (*Paracoccidioides brasiliensis* from the respiratory system, and *Candida lactis-condensi*, *Oosporidium margaritiferum*, *Rhodospo - ridium diobovatum* and *Rhodosporidium kratochvi-lovae* from the digestive system); and that Dynowska et al. [23] managed to isolate as many as 6 species of the genus *Trichosporon* from the gastrointestinal tract of oncological patients. Two of these, i.e. *Trichosporon asahii* and *Trichosporon mucoides,* have not been previously recorded in Poland. The results referring to the gastrointestinal tract are extremely important in the context of interactions with bacteria, *Helicobacter pylori* in particular [4,25,27]. In the respiratory system, bacteria that often co-operate with fungi include *Staphylococcus aureus* and *Streptococcus pneumoniae* (current results are being analyzed). The appearance of a species typical of moist, tropical forests of the Middle America, i.e. *Paracoccidioides brasiliensis*, in the materials that we analyzed suggests enhanced intercontinental migration of fungi linked with man. This species may be a causative agent of paracocci-dioidomycosis, a chronic disease whose route of invasion has not been recognized to date. Most likely, this pathogen penetrates through the respiratory system or through erosions at local

injuries. It may indicate stomatitis (leading to the loss of teeth) often connected with enlargement of lymph nodes – in the course of the disease, systemic lesions are induced in the lymphatic and digestive systems, spleen, lungs and liver [13,28]. In addition, this fungus displays special affinity with mucous membranes. In the host's tissues it occurs in the form of yeast-like budding cells.

The successful isolation of so many fungi from different biological sources from human subjects is attributable not only to the highly advanced laboratory diagnostics that were employed but – most of all – a resultant of the rate of increase in and accumulation of iatrogenic and environmental factors that predispose for mycotic infections and successively impair the human body's resistance to these infections.

To conclude, the respiratory and digestive systems should be treated not only as a reservoir of potential anthropopathogens but also as a route of invasion leading to proliferation of fungi, often leading to fungemia [4,20,25]. Any changes in the diversity and prevalence of fungi in ontocenoses of these systems indicate habitat changes and inform which fungi are currently predominating in the habitat [5,6,24]. This information is of great significance because of the increasing number of factors predisposing for fungal infections, and it remains unknown when, and under which precise conditions, the symbiotic commensal transforms into the invasive parasitic form.

Conclusions

It is necessary to continue long-term mycological monitoring (environmental and individual) of people with no earlier history of fungal infections, when the appearing symptoms indicate mycotic infections or when etiological factors other than fungi have already been excluded.

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Wyniki wieloletnich analiz mikologicznych materiałów biologicznych pochodzących z wybranych ontocenoz narządowych – drożdże i grzyby drożdżopodobne

M. Dynowska, K. Góralska, P. Troska, G. Barańska, A. Biedunkiewicz, E. Ejdys, E. Sucharzewska

Praca jest obszernym fragmentem badań monitoringowych i obejmuje wyniki 20-letnich obserwacji, dotyczących dynamiki i zróżnicowania gatunkowego grzybów najczęściej zasiedlających układ oddechowy i pokarmowy. Materiał do badań stanowiły wymazy i wycinki z przewodu pokarmowego, wymazy z jamy ustnej i gardła, plwocina oraz płyn bronchoskopowy z układu oddechowego. Materiał biologiczny poddano rutynowej diagnostyce mikologicznej, oznaczono i przyporządkowano taksonomicznie.

Ogółem wyizolowano 41 gatunków grzybów: 34 z układu oddechowego i 25 z układu pokarmowego. W ostatnim 10-leciu odnotowano bardzo wyraźny wzrost liczebności grzybów, zwłaszcza w układzie pokarmowym. Aż 18 gatunków izolowano z obydwu układów – dominowały wśród nich grzyby z rodzaju *Candida* i ich formy doskonałe. Na uwagę zasługuje częste notowanie drożdży właściwych (*Saccharomyces* spp.) oraz stwierdzenie w układzie oddechowym endemicznego gatunku *Paracoccidioides brasiliensis*, a w układzie pokarmowym stadiów płciowych (*Rhodosporidium diobovatum*, *Rhodosporidium kratochvilovae*).

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