Journal of Bacteriology & Parasitology

Góralska et al., J Bacteriol Parasitol 2016, 7:3
http://dx.doi.org/10.4172/2155-9597.1000279

Research Article Open Access

Human Mycobiota-Selected Ontocenoses of Students of Natural Science and Medicine

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Received date: June 02 08, 2016; **Accepted date:** June 25, 2016; **Published date:** June 29, 2016

Abstract

Objectives: The aim of this study was to compare the prevalence of yeast-like fungi and yeasts on the mucous membranes of a group of students of Natural Sciences and Medicine.

Materials and methods: The study involved 156 students of the Faculty of Biology and Biotechnology (FB&B) and 37 students of the Faculty of Medical Sciences (FMS). Material samples were collected with sterile cotton swab from the nose, mouth and throat. The identification of fungi was carried out by the evaluation of macrocultures and microcultures on Nickerson agar and biochemical features.

Results: Yeast-like fungi and yeast were isolated from 41.97% of the subjects (81 people). The fungal colonization of the mucous membrane was observed in 59 students of the Faculty of Biology and Biotechnology (37.82%) and 22 students (56.46%) of the Faculty of Medical Sciences. The obtained fungi were classified into 31 taxonomic units. The predominant species were *Candida dubliniensis* and *Lachancea thermotolerans* (syn. *Kluyveromyces thermotolerans*). 14 species with defined status of BSL were recorded.

Conclusions: Yeast and yeast-like fungi were more frequent among students of Biology and Biotechnology, than students of Medical Sciences. In students of FB&B greater taxonomic diversity of fungi was found than in students of FMS. The species diversity and prevalence of fungi observed in students of the Life Sciences are determined not only by lifestyle, but mainly by the possibility and frequency of contact with a variety of reservoirs and sources of potentially pathogenic fungi. The occurrence in human ontocenoses of 14 species classified to 1st and 2nd class of Biosafety Level is also very important.

Keywords: Yeast; Oral cavity; *Candida*; Kluyveromyces; Human mycobiota

Introduction

Fungal infections represent a growing clinical and therapeutic problem which mainly concerns patients with significantly compromised immune systems. In many hospital wards, such as Oncology, they cause many complications, often leading to death. Most infections are endogenous, and the oral cavity is the most common reservoir of fungi. In the human body fungi belonging to different taxonomic groups, both yeast as yeast-like fungi, were found. Yeast can be defined as fungi belonging to the class *Ascomycota*, multiplying asexually by budding, and sexually forming spores of ascospores type. While, a group of yeast-like fungi include the fungi belonging to the class *Basidiomycota*, proliferating asexually by budding, but forming sexual spores of basidiospores type, or fungi proliferating exclusively asexually by budding, for which sexual form is unknown.

The most common fungi in human organism are of the genus *Candida*, and the etiological agent is mainly *Candida albicans*, found in up to 70% of patients [1-5]. Other frequently encountered species are *Candida glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. Noteworthy is *Candida dubliniensis*, whose prevalence in Poland has increased significantly since it was first recorded in 1995 [6,7]. *C. dubliniensis* is often confused with *C. albicans* and relatively rarely found, in 3.5% of healthy and 30% of HIV-infected patients with a weak immune system. While in patients with respiratory diseases and digestive system ailments, its prevalence is recorded as 8-9% [5].

Students may be more predisposed to fungal colonization due to their lifestyle: lack of a regular and varied diet, too much time spent indoors during classes and lectures, too short a time for physical activity and rest, and staying in places of higher population density, such as dormitories, classrooms and laboratories, which may lead to inter-transmission of fungi [8,9]. Not without the meaning is also the way how students spend their free-time-lack of sleep, parties, web social nets etc.

An increasing number of fungal infections in patients belonging to risk groups and the increased prevalence of yeast-like fungi and yeasts in healthy subjects suggest the need for continuous mycological monitoring of various groups. For individuals with an increased risk of mycoses belong, among others oncological patients, treated with radiation and chemotherapy, HIV infected persons, patients with neutropenia, as well as in the course of prolonged treatment with antibiotics or steroids. Disorders of proper functioning of the immune system and changes in the structure and abundance of natural

microbiota contribute to the onset and development of fungal infection. Medical staff constitutes one of the main reservoirs for microorganisms responsible for the occurrence of nosocomial infections, as well as their main vectors. In the course of occupational practice and future profession medical students will take care of patients of risk groups, and therefore, fungal colonization of the mucous membrane of students can promote the spread of fungal infections in patients. In addition, students of the Natural Sciences often come into contact with a variety of biological materials, including plant and animal tissues that may have been contaminated with fungi. Most biotechnological experiments require high standards of sterility and the presence of fungi in laboratory staff may contribute to contamination of experimental material, resulting in damaged material and lost reagents, or false results caused by the formation of metabolites which may change the physical and chemical characteristics of analyzed compounds. The aim of this study was to determine the prevalence and the variety of yeast-like fungi and yeasts on the mucous membranes of a group of students of Natural Sciences and Medicine.

Materials and Methods

The study involved 156 students of the Faculty of Biology and Biotechnology (FB & B) and 37 students of the Faculty of Medical Sciences (FMS). All students were volunteers, declared good health, average physical activity and proper oral hygiene. They have never been treated in the direction of fungal infection and there were no changes in their oral mucosa. The diagnostic scheme recommended by Dynowska [10] was used. Material samples were collected with sterile cotton swab from the nose, mouth and throat, inoculated into solid Sabouraud medium (BTL) and incubated at 37°C for 24-48 hours. The obtained fungal cultures were passaged several times on Sabouraud slants with chloramphenicol and gentamycin, to proliferate and produce clean isolates, without bacteria (Photo 1). The isolates were evaluated macroscopically on Sabouraud (BTL) and CHROMagar (GRASO) media. Microscopic evaluation was performed on the Nickerson medium (BTL) supplemented with biotin and trypan blue. Biochemical properties, such as fermentation of sugars was assessed on liquid medium supplemented with bromocresol purple and methylene blue, and the assimilation of carbon compounds on the assimilation medium containing magnesium and potassium salts. Classification of fungi to a species was performed using special keys: De Hoog et al. [11] and Kurtzman and Fell [12] and Kurtzman et al. [13]. Statistical analysis was performed with t-Student test by using STATISTICA 11.0 software.

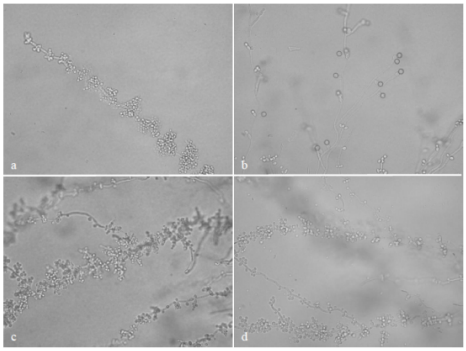

Photo 1: Microcultures on Nickerson agar (mag. 400x): a) *C. albicans*, b) *C. dubliniensis*, c) *M. guilliermondii*, d) *C. tropicalis*.

Results

Yeast-like fungi and yeast were isolated from 41.97% of the subjects (81 people). The fungal colonization of the mucous membrane was observed in 59 students of the Faculty of Biology and Biotechnology (37.82%) and 22 students (56.46%) of the Faculty of Medical Sciences. Fungi were isolated from the oral cavity of 75 patients. Ontocenotic yeasts were isolated from the noses of 4 students, and the throats of 13. Only in 10 cases fungi were recorded in two ontocenoses simultaneously (Table 1). There were no differences in the prevalence of fungi, depending on the age and gender of students tested.

No.	Species/Taxa	Number of isolates		Biosafety Level Class
		Faculty of Biology and Biotechnology	Faculty of Medical Sciences	
1	*Candida albicans var. stellatoidea*	1	0	2
2	*Candida dubliniensis*	13	0	2
3	*Candida fennica*	1	0	-
4	*Candida glabrata*	0	1	2
5	*Candida glaebosa*	1	0	-
6	*Candida ishiwadae*	1	0	-
7	*Candida lipolytica*	0	1	2
8	*Candida parapsilosis*	1	3	2
9	*Candida tropicalis*	2	5	1/2
10	*Citeromyces matritensis*	1	0	-

11	*Cystofilobasidium capitatum*	0		1		-
12	*Debaryomyces hansenii*	2		1		1
13	*Debaryomyces robertsiae*	1		0		-
14	*Dekkera anomala*	4		0		1
15	*Dipodascus armillariae*	1		0		-
16	*Kluyveromyces lactis*	6	8	0	0	1
17	*Kluyveromyces lactis var. drosophilarum*	2		0		
18	*Kluyveromyces marxianus*	4		7		2
19	*Lachancea cidri*	1		0		-
20	*Lachancea thermotolerans*	7		6		-
21	*Magnusiomyces ingens*	2		0		-
22	*Metschnikowia pulcherrima*	1		0		2
23	*Meyerozyma guilliermondii*	0		2		2
24	*Saccharomyces cerevisiae Meyen*	3		0		1/2
25	*Saccharomycopsis capsularis*	3		2		-
26	*Saccharomycopsis vini*	0		1		-
27	*Schwanniomyces occidentalis var.*	5		0		-
28	*Schwanniomyces polymorphus var.*	4		1		-
29	*Zygosaccharomyces bisporus* H.	1		0		-
30	*Zygosaccharomyces rouxii*	1		0		2
31	Species of *Divisio Basidiomycota*	2		0		-

Table 1: Species of fungi obtained from students of University of Warmia and Mazury in Olsztyn.

In total, 92 isolates were obtained: 82 single-species and 10 two-species. The obtained fungi were classified into 31 taxonomic units. The predominant species were *Candida dubliniensis* and *Lachancea thermotolerans* (syn. *Kluyveromyces thermotolerans*), both species occurred at a frequency of 12.75%. *Kluyveromyces marxianus* was slightly less prevalent with 10.7%. *Kluyveromyces lactis* represented 9.8% of positive samples, and *Candida tropicalis*-6.86% (Figure 1).

Differences in the occurrence of taxa were found in students of both faculties. In students of the FB & B, 65 isolates of 26 species were found, among which *Candida dubliniensis* clearly dominated (18.31%). Also, *Kluyveromyces lactis* (11.27%) and *Lachancea thermotolerans* (9.86%) were often recorded. While from FMS students, 27 positive cultures belonging to 12 taxa were obtained (Figure 1). The most frequently isolated were *Kluyveromyces marxianus* and *Lachancea thermotolerans* (19.35% each), followed by *Candida tropicalis* (16.13%). There was statistically significant difference between biodiversity and abundance of species in both groups of students ($p<0.05$).

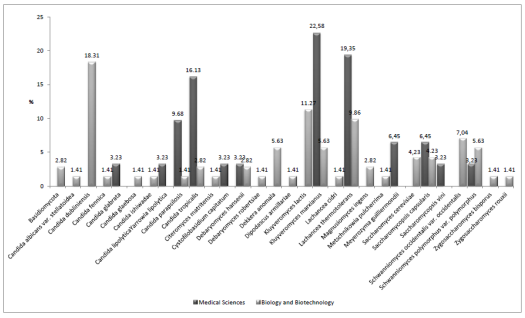

Figure 1: Frequency [%] of fungi isolated of oral cavity of carrier-students of Faculty of Medical Sciences and Faculty of Biology and Biotechnology.

Nineteen species found in FB&B students were not found in materials derived from FMS students including *Candida dubliniensis*, which was very frequently found, and *Kluyveromyces lactis*, slightly less so, also *Schwanniomyces occidentalis* var. *occidentalis* (syn. *Debaryomyces occidentalis*) and *Dekkera anomala* were not recorded.

However, 6 species found in students of Medical Sciences, including *Candida glabrata*, *Candida lipolytica*, *Candida pseudotropicalis*, *Cystofilobasidium capitatum*, *Meyerozyma guilliermondii* (syn. *Pichia/Candida guilliermondii*) and *Saccharomycopsis vini* were not obtained from FB & B students. One species – *L. thermotolerans* was identified with statistically significant higher prevalence in both students groups ($p<0.05$). Only seven species were recorded in both groups of students, including 4 species with defined status of BSL (Figure 2). In materials derived from FB&B students there was 8 species classified to BSL 2 and 3 to BSL 1 group, while among materials from FMS 7 of BSL 2 and 1 of BSL 1. Differences were statistically significant ($p<0.05$).

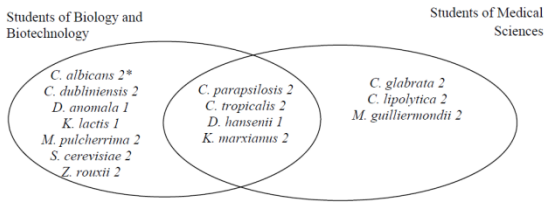

Figure 2: Species with defined BSL status isolated from students of Medical Sciences and Biology and Biotechnology (*BSL status).

Discussion

Oral colonization by yeasts is not always a sign of an ongoing disease process. Contemporary human is exposed to a number of factors disrupting homeostasis. Stress, lack of physical activity, clothes made of synthetic materials, and a high-carbohydrate 'fast food' diet are conducive to lowering immunological barriers and therefore inviting colonization by fungi [2,14,15].

Literature data indicates a prevalence of fungi in the oral mucous membrane as high as 96% [1,3,7,16-18]. The results of the present study correspond with examples of literature data concerning healthy adults, but in a different age group: the closest match being those of Zaremba et al. [19] obtained in adults between 56 and 70 years of age (35%). The same study notes a much higher prevalence of fungi in younger patients, aged 35-44 (67%) and above 70 years of age (74%). In total, fungi were found in 63% of the surveyed [19]. Similar results were obtained by Loster et al. [20], who observed yeasts in 63.3% of generally healthy subjects using acrylic dentures. A similar proportion of carriers, 70%, was reported in patients with impaired function of salivary glands, which contributed to the drying of the oral cavity mucous membrane [3]. These patients had a significant share of mixed isolates: 37.5% of two-species and 9.5% of three-species.

In the present study, fungi were recorded slightly less frequently than in studies by Biedunkiewicz [4] conducted in a population of students of Biology and Veterinary Medicine of a comparable age. Author observed fungi in 48% of surveyed students in the autumn, and in 46% in the spring. The prevalence of fungi in students of Medical Sciences was similar to that recorded by Bonassoli and Svidzinski [21] in nursing students. Of the 22 students examined, as many as 15 were found to be colonized by yeasts, 68%. Also studies conducted in Medical University of Lodz [2] showed the prevalence of fungi in oral cavity of students on the level 68%. Higher prevalence was noticed with respect to the age of the examined individuals. In a study conducted by Majima et al. [22] among dental students was found a significantly lower prevalence of fungi in the oral cavity, at 18.7%. Bonassoli and Svidzinski [21] suggest that the high prevalence of fungi in subjects related to health care, among others nurses, physicians and medical students, can promote the spread and occurrence of fungal infections among patients, especially with impaired immune system function. Previous studies indicate that this relationship may be mutual, that is, the hospital environment can be a source of infection for staff and apprentices/trainees, evidenced by the fact that a much higher prevalence of fungi, but with less diversity, were found among FMS students than FB&B students. However, a large number of the taxa obtained from the FB&B students may be associated with exposure to a variety of biological material - plant tissue, animal and the habitats of organisms from different taxonomic groups. Hence, the mucous membrane of Natural Sciences students may be colonized by species less common in the human population, but typical to other organisms from aquatic and terrestrial environments.

The dominant species reported in previous studies of the mucous membrane was *Candida albicans*, isolated from 60-89% of subjects [17,18,22-24]. Other species were recorded with significantly lower incidence: *C. parapsilosis* in 33% and *C. tropicalis* only in 13% of patients [2,3,20,21,25-27]. In recent years, the proportion of *C. albicans* has declined in favor of other yeast-like fungi species. Biedunkiewicz [4] isolated *Candida tropicalis* (41%) slightly more often from students than *Candida albicans*: 39% of positive samples. Also, yeasts, other than *C. albicans*, dominated in the present study: *Candida dubliniensis* – a species more often found in organ ontocenoses and *Lachancea thermotolerans* (syn. *Kluyveromyces thermotorelans*), used in fermentation technology [6,28,29].

A larger diversity of fungal species were found in the present study in comparison with literature data. A significant proportion comprised species such as *Metchnikowia pulcherrima*, *Dekkera anomala* and *Lachancea thermotolerans*, which are rare or very rarely listed in materials of human origin. Species considered by most authors as dominant in human microbiota either represented a small percentage of isolates in the present study or were not found. From the students examined were isolated fungi of genera: *Lachancea*, *Schwanniomyces* and *Zygosaccharomyces*, which until now were not mentioned in the medical literature as associated with the human organism. Clear trends regarding changes in the taxonomic structure of the human mycobiota can be seen in the mycological literature of the last 10-20 years [17,18,28,30]. The prevalence of previously dominant species has decreased and new species have taken their place, and these are often much more aggressive to the human body. These observations are consistent with the principle of ecological succession observed in natural environments.

Studies conducted by Romanowska-Tołłoczko [9] show that most students do not lead a healthy lifestyle: lack of physical activity, alcohol consumption, cigarette smoking and a diet with little variety. According to Soysa and Ellepola [31], tobacco smoking promotes the increase of glucose in the saliva and the reduction of leukocytes and immunoglobulin activity in the oral cavity, and aromatic hydrocarbons present in the tobacco may be a source of nutrients for fungi [32]. Studies conducted among medical students have shown that consumption of large amounts of sugary drinks, especially with exceeding GDA of sugar, can significantly increase the incidence of fungi in the oral cavity [2]. Therefore, it is important to maintain proper eating habits and a healthy lifestyle in antifungal prophylaxis [33-35].

The results of the conducted analysis confirm those of other current studies addressing the characteristics of a healthy human fungal microbiome [36]. As suggested by own studies, the species diversity and prevalence of fungi observed in students of the Life Sciences are determined not only by lifestyle, but mainly by the possibility and frequency of contact with a variety of reservoirs and sources of potentially pathogenic fungi. The great importance has also the occurrence in human ontocenoses of 14 species classified to 1st and 2nd class of Biosafety Level. Class BSL 1 includes fungi which can be responsible for mild superficial infection and BSL 2 gathered fungi causing opportunistic infections mostly in individuals with weakened immune system [37]. In the context of nosocomial infections occurrence of fungi with determined Biosafety Level class on mucus membranes of future health care workers can have the great meaning for epidemiological chain.

Acknowledgements

Research funded by the Department of Mycology (University of Warmia and Mazury in Olsztyn).

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