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ORIGINAL RESEARCH PAPER

Non-lipophilic mycobiota of human skin

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Abstract
The human skin is inhabited by many species of bacteria and fungi, which are its natural microbiota. Fungi colonizing the skin, including those causing disease, characterized by great variety and variability, can be influenced by various factors. The purpose of this study was to investigate the composition of the non-lipid-dependent fungal microbiota of skin, including the presence of species potentially pathogenic for humans. Fifty-six volunteers of both sexes aged 22–78 were subjected to the study. Swabs were taken from the face, chest, back and interdigital spaces of hands. Mycobiota isolated proved to vary both in terms of the location of occurrence and gender of patients. Interdigital spaces of hands, dominated by yeasts, constitute a location on human skin most contaminated with fungi. Molds were more often isolated from the face and chest. The back was the least contaminated location. There was no difference in fungal incidence in relation to sex.

Keywords
mycobiota; yeasts; microbial diversity; molds

Introduction

The human skin is inhabited by many species of bacteria and fungi, which are its natural microbiota. These organisms perform many beneficial functions. Among others, through competition, they do not allow pathogenic species to colonize the skin [1,2]. Mycobiota of skin is dominated mainly by lipophilic fungi of the genus *Malassezia*. Additionally, other species of yeast, mainly of the genus *Candida* and *Cryptococcus*, may exist on the skin. Species composition of skin mycobiota is characterized by great diversity and variability, which can be influenced by various factors such as age, occupation, and the climate of the place of residence [3–5].

Monitoring of currently dominant fungal species belonging to the natural microbiota as well as the frequency and location of infection caused by these fungi is important for epidemiological studies. Collecting these data allows drawing conclusions about ongoing changes and forecasting changes to mycobiota in the near future.

The aim of this study was to determine settlement of human's skin by non-lipophilic fungi.

Material and methods

The study involved 56 generally healthy (no responding symptoms of fungal infection of skin and antifungal therapy) volunteers (41 women and 15 men) aged over 18 years. The mean age was 43.7 for women ±13.5 years (range 22–78 years, median = 42 years), and for men 48.9 ±15.7 years (range 17–75, median = 50).

The study was conducted in 2012–2014. Swabs were taken from nasolabial folds, the area of the breastbone, the back and interdigital spaces of hands for every study participant. Then the swabs were immediately cultured on Sabouraud glucose agar (SGA) with chloramphenicol and incubated at 27°C for 2–3 weeks and periodically checked.

The growing colonies were subcultured on appropriate media (rice agar for yeasts and sporulation enhancement medium for molds, i.e., Czapek–Dox agar). The fungal identification to the genus level was based on identification keys according to *Atlas of clinical fungi* (2nd ed.) [6]. For yeasts, in some cases, species identification was confirmed by API 20C AUX strips (bioMérieux) according to manufacturer's procedure. For the mold colony, we performed adhesive tape slides in lactophenol cotton blue. In cases in which this method was not enough to ID genera, we performed slide cultures. For molds colony we perform scotch type slides in lactophenol cotton blue in cases that this method was not enough to ID genera we perform slide culture.

The study was approved by the Bioethical Committee of Jagiellonian University (No. KBET/164/B/2012).

Statistical analysis was performed using R Language and Environment for Statistical Computing software [7]. The significance level for all statistical tests was set at alpha (p) ≤ 0.05.

Results

A positive culture from at least one location was obtained from 38 participants (n = 27; 65.8% of women and n = 11; 73.3% of men). There were no differences in the overall incidence of colonization between the sexes (Fisher's exact test, p = 0.7508). Altogether, 72 (22.6%) positive cultures were obtained from 223 swabs (36 strains of yeast-like fungi and 30 of molds; 10 cultures showed mixed mycobiota – yeasts and molds). No fungi were isolated from any location in 18 participants. In 19 patients, a few species of fungi were isolated simultaneously from a single location. Further results are given in Tab. 1.

Tab. 1 Frequency of isolation of fungi from different locations on the skin depending on the gender of the test participants.

		Face		Chest		Back		Hands	
Gender	Group of fungi	*n**	%	*n*	%	*n*	%	*n*	%
F	Lack of fungi	25	62.50	26	63.41	31	75.61	23	63.41
	Yeasts	6	15.00	6	14.63	5	12.20	13	14.63
	Molds	9	22.50	7	17.07	3	7.32	1	2.44
	Mixed	0	0.00	2	4.88	2	4.88	4	9.76
M	Lack of fungi	11	73.33	11	73.33	13	86.67	7	46.67
	Yeasts	3	20.00	0	0.00	1	6.67	2	13.33
	Molds	1	6.67	4	26.67	1	6.67	4	26.67
	Mixed	0	0.00	0	0.00	0	0.00	2	13.33
F+M	Lack of fungi	36	65.45	37	66.07	44	78.57	30	53.57
	Yeasts	9	16.36	6	10.71	6	10.71	15	26.79
	Molds	10	18.18	11	19.64	4	7.14	5	8.93
	Mixed	0	0.00	2	3.57	2	3.57	6	10.71

F – female; M – male; n – number of samples. * One participant refused to take a swab from her face because of her makeup.

The most frequently cultured yeasts in all locations were representatives of the genus *Candida*. Among the molds, the most popular kinds were *Aspergillus*, which was isolated from swabs from the face and back, and *Penicillium* isolated from the chest and smears from interdigital spaces of hands. One participant was found to be colonized by one species (*Candida lusitaniae*) at all test sites on the skin.

Fungi were usually isolated from the interdigital spaces of fingers (n = 26, 43%), and least often from the back (n = 12, 18% of participants; Fig. 1).

There were no differences in the frequency of isolation of fungi on the grounds of sex

in relation to isolation location (Cochran–Mantel–Haenszel test, $p = 0.8371$). However, after grouping the results for both sexes, it was found that the incidence of fungi depending on location is significantly different (chi-square test, $p = 0.01191$). In order to find a relationship between the location and the isolated fungi, correspondence analysis was carried out, the results of which are shown in Fig. 2. Therefore, it can be concluded that the occurrence of yeasts is associated with interdigital spaces, while the face and chest are associated with a higher incidence of molds. The back turned out to be the location from which fungi were isolated least frequently.

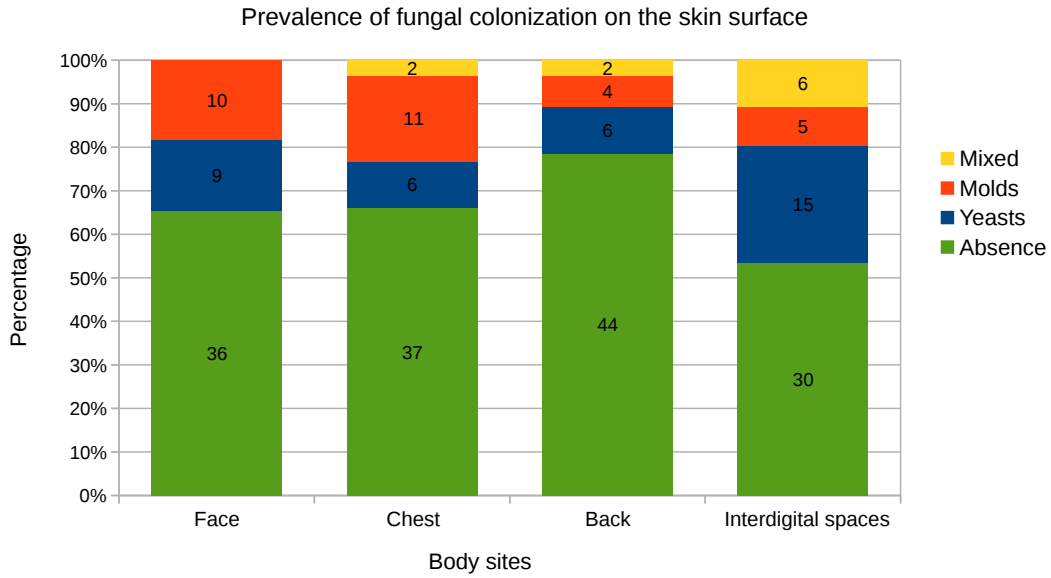

Fig. 1 The incidence of fungal colonization depending on the skin location.

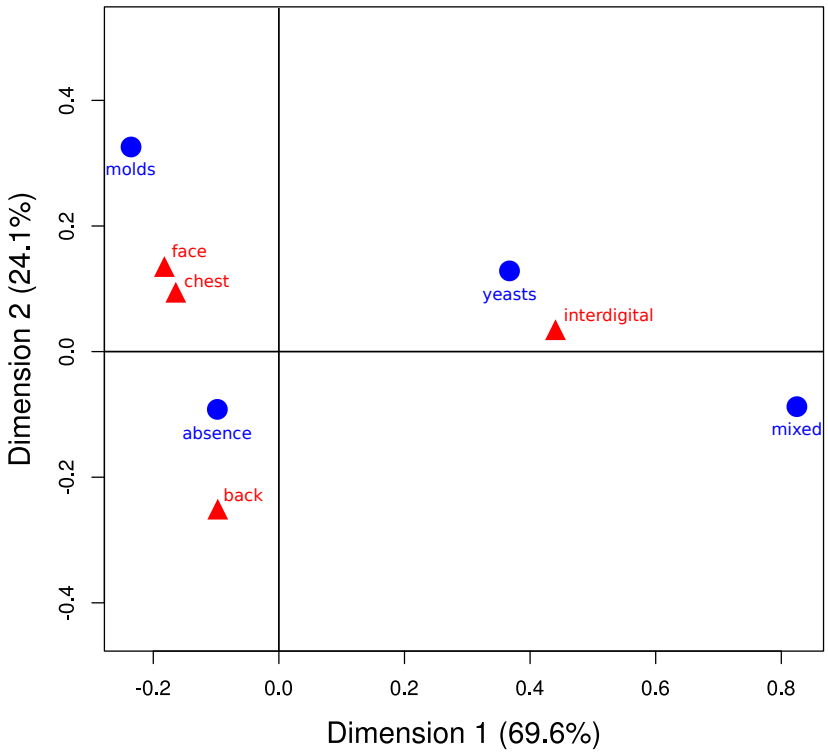

Fig. 2 Correspondence analysis: the presence of fungi and location. The blue indicates the location; the red indicates fungal group.

Discussion

There are no effective methods to assess the actual presence of fungi on the skin. Culture methods have their limitations associated with the lack of providing all essential conditions for growth for each species of mycobiota in vitro. It appears that the solution to this problem could be the application of molecular techniques. However, interpretation of the occurrence of species based on the isolation and sequencing of DNA in projects investigating the human microbiota is also problematic. The mere discovery of fungal DNA does not mean that an entire cell is present. And even if a fungal cell is present, it is not possible to assume that it is alive. Another very important problem is widespread contamination of laboratory equipment such as reagents, test tubes, swabs, etc. with microbial DNA [8].

Furthermore, interpretation of the results of cultures and the results of molecular studies causes a number of problems mainly due to the fact that occasional isolation or finding the presence of fungal DNA do not mean that a particular species colonizes the skin, it could be transient. Fungi on the skin may occur accidentally as a result of contamination with soil, plant remnants, or after exposure to water or air. Many fungi, including molds, are anemochorous; therefore, their spores are commonly airborne and may passively fall on the skin. This is confirmed by our findings indicating a lower incidence of fungi on the covered skin of the back and the highest prevalence in interdigital spaces which are exposed not only to the atmosphere but also to tap water and all kinds of contamination being the origin or the breeding ground for fungi. Research of the mycobiome, independent of culture, showed that, apart from the DNA of *Malassezia*, the most common was the DNA of *Penicillium* and *Aspergillus* molds [3]. Also in the present study, based on culturing methods, these two kinds of fungi were predominant.

Most fungi were isolated from interdigital swabs. In the report entitled *Environmental and hand hygiene of the Polish people*, published by Dettol and Children's Memorial Health Institute [2], fungi were isolated in women from 17 (15 yeasts and 2 molds) out of 636 samples and in men from 10 (9 yeasts and 1 mold) out of 550 samples. The study suggests that there are no differences in the incidence of yeasts between the sexes (chi-squared test with Yates' correction for continuity, $p = 0.5004$, own calculations). Our study also failed to demonstrate a more frequent colonization of interdigital spaces by yeasts in women than in men; it was approximately 14% for each group (Fisher's exact test, $p = 0.3064$).

In this respect, it is curious that there is a higher incidence of candidiasis of interdigital spaces of hands and candidiasis of fingernails in women [9]. Saprotrophic yeasts have similar life requirements to the requirements of the pathogen called *Candida albicans*. It is known that *Candida albicans* is usually found on the skin. Other *Candida* species that are predominant there are *C. tropicalis*, *C. parapsilosis* and *C. orthopsilosis* [3]. Following deliberate inoculation of hands, after 45 minutes, *Candida parapsilosis* showed greater survivability (*C. albicans* was also cultured in an amount of around 2.5–5% initial inoculum) [10]. The question arises of whether an earlier colonization by other species of yeasts is a safeguard against or predisposes people to infections with *C. albicans*.

The presence of *C. albicans* on the skin of hands is usually associated with poor hygiene. These fungi live in the gastrointestinal tract and mucous membranes of the reproductive organs, from where they can be easily transferred to one's hands, particularly when using the restroom. As reported in studies of the American Society for Microbiology, women wash their hands more often (75–93%) than men (58–77%) after using the restroom. In Great Britain, this is only 32% of men and 64% of women. These figures are somehow contrary to a higher incidence of mycosis in women. It is possible that mere washing without the use of disinfectants could also give rise to colonization. As was shown by Biedunkiewicz and Schulz's research, 66% of tap water samples contained fungi, of which the most numerous were yeasts (81.3% of the isolated fungi) [1]. Moreover, it is known that detergents damage the protective lipid layer of the skin [11], which, together with maintaining skin's moisture (also through creams and emollients), may favor colonization and, consequently, infection.

The problem of the presence of fungi on healthy skin and associating colonization with potential development of infection needs further investigation. One must

remember that the promoted molecular testing techniques do not allow us to assess the presence and viability of cells and merely finding the presence of a particular species, whether with the use of molecular methods or culturing, does not mean that the given species is a permanent component of skin mycobiota.

Conclusions

- Human skin locations most contaminated with fungi are interdigital spaces of hands.
- Interdigital spaces of hands are dominated by yeasts, whereas molds are prevalent on the face and chest. However, there were usually no fungi isolations from the back.
- There is no difference in the incidence of fungi depending on gender.

References

1. Biedunkiewicz A, Schulz Ł. Fungi of the genus *Exophiala* in tap water. Mikologia Lekarska. 2012;19:23–26.
2. Raport marki Dettol i Centrum Zdrowia Dziecka. Higiena rąk i otoczenia Polaków [Internet]. 2007 [cited 2016 Jan 21]. Available from: http://pis.lodz.pl/data/other/hr_polacy.pdf
3. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, et al., Topographic diversity of fungal and bacterial communities in human skin. Nature. 2013;498(7454):367–370. http://dx.doi.org/10.1038/nature12171
4. Hannigan GD, Grice EA. Microbial ecology of the skin in the era of metagenomics and molecular microbiology. Cold Spring Harb Perspect Med. 2013;3(12):a015362. http://dx.doi.org/10.1101/cshperspect.a015362
5. Pawłowicz A, Adamski Z. Flora dermatofitowa i oportunistyczna w zmianach grzybiczych dłoni, stóp oraz paznokci u pacjentów Kliniki Dermatologii AM w Poznaniu w latach 1984-1994. Mikologia Lekarska. 1994;2:95–100.
6. de Hoog GS, Guarrao J, Gene J, Figueras MJ. Atlas of clinical fungi. 2nd ed. Centraalbureau voor Schimmelcultures Utreht. Reus: Universitat Rovira i Virgili; 2000.
7. R Core Team. A language and environment for statistical computing [Internet]. Vienna: R Foundation for Statistical Computing; 2013 [cited 2016 Jan 21]. Available from: http://www.r-project.org
8. Salter S, Cox MJ, Turek EM, Calus ST, Cookson WS, Moffatt MF, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014;12:87 http://dx.doi.org/10.1186/s12915-014-0087-z
9. Kwaśniewska J, Szefer E, Jaskółowska A. Niektóre aspekty grzybic skóry i paznokci na podstawie danych pacjentów zakładu diagnostyki i leczenia chorób pasożytniczych i grzybic uniwersytetu medycznego w Łodzi. Mikologia Lekarska. 2012;19(1):17–22.
10. Rangel-Frausto MS, Houston AK, Bale MJ, Fu C. Wenzel RP. An experimental model for study of Candida survival and transmission in human volunteers. Eur J Clin Microbiol Infect Dis. 1994;13(7):590–595. http://dx.doi.org/10.1007/BF01971311
11. Okuda M, Yoshiike T, Ogawa H. Detergent-induced epidermal barrier dysfunction and its prevention. J Dermatol Sci. 2002;30(3):173–179. http://dx.doi.org/10.1016/S0923-1811(02)00106-8