

THE INFLUENCE OF DIET ON GASTROINTESTINAL *CANDIDA* SPP. COLONIZATION AND THE SUSCEPTIBILITY OF *CANDIDA* SPP. TO ANTIFUNGAL DRUGS

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ABSTRACT

Background. *Candida* spp. has been identified as the most common member of human gut microbiota. This yeast-like fungus is recognized as an opportunistic organism due to its potential to cause diseases in all parts of the gastrointestinal tract.

Objective. The aim of the study was to investigate the relationship between diet and health of the study participants and the presence of gastrointestinal *Candida* spp. Susceptibility of *Candida* to antifungal drugs was determined.

Material and methods. Material comprised of oral swabs and fecal samples self-collected by the study participants. The participants filled in a nutritional questionnaire. A total of 30 people took part in the study, including 28 women and 2 men. The study was conducted in Wrocław, Poland. Susceptibility of *Candida* to antifungal drugs was determined using Bio-Rad's FUNGITEST™ Kit designed for the susceptibility testing of yeasts to six antifungal drugs.

Results. In the group with negative *Candida* spp. cultures, healthier wheat flour substitutes were consumed significantly more frequently than in the group with positive *Candida* spp. cultures. Yellow cheeses and quark were eaten significantly more frequently in the group with negative *Candida* spp. cultures. No antifungal resistance was detected in the study group.

Conclusions. The increased consumption of purified wheat flour products was linked to the presence of gastrointestinal *Candida* spp. A higher consumption of cheese was observed in the group with negative *Candida* spp. cultures, which may indicate the inhibitory effect of saturated fatty acids on the growth of human *Candida* spp. Sensitivity of *C. albicans* to antifungal drugs may increase effectiveness of candidiasis treatment.

Key words: *Candida* spp., diet, fecal samples, antibiotics, drug resistance

STRESZCZENIE

Wprowadzenie. *Candida* spp. to najczęstszy element mikroflory przewodu pokarmowego. Ten grzyb drożdżopodobny jest oportunistą, ze względu na możliwość powodowania chorób we wszystkich odcinkach przewodu pokarmowego. Pomimo, że *Candida* spp. jest jedną z głównych przyczyn zakażeń oportunistycznych, to istnieje ograniczona liczba środków przeciwygrzybiczych stosowanych w terapii. Szerokie zastosowanie antybiotyków spowodowało zwiększenie oporności szczepów na stosowane leki.

Cel. Celem pracy było zbadanie zależności między dietą i stanem zdrowia osób biorących udział w badaniu, a obecnością *Candida* spp. w przewodzie pokarmowym. Ponadto oceniono wrażliwość wyizolowanych szczepów *Candida* spp. na najczęściej stosowane leki przeciwygrzybicze.

Materiał i metody. Materiałem do badania były wymazy z jamy ustnej i próbki kału, pobrane samodzielnie przez badanych. Badani wypełniali ankietę dotyczącą częstotliwości spożycia różnych grup produktów spożywczych oraz występowania czynników ryzyka kandydozy. Przebadano 30 osób, w tym 28 kobiet i 2 mężczyzn. Badanie przeprowadzono na terenie miasta Wrocław, w Polsce. Oznaczono wrażliwości uzyskanych *Candida* na leki przeciwygrzybicze z użyciem zestawu FUNGITEST firmy Biorad, który umożliwia badanie wzrostu drożdżaków w obecności 6 preparatów przeciwygrzybiczych.

Wyniki. Wykazano dodatnią korelację pomiędzy spożyciem produktów z oczyszczonej mąki pszennej, a występowaniem *Candida* spp. w przewodzie pokarmowym. Zaobserwowano wyższe spożycie serów w grupie osób z wynikiem ujemnym badania w kierunku *Candida* spp., co może wskazywać hamujący wpływ nasyconych kwasów tłuszczowych na wzrost *Candida* spp. w organizmie. Wśród badanych szczepów nie wykryto opornych na leki przeciwygrzybicze.

Wnioski. Duże spożycie produktów z pszennej, oczyszczonej mąki było związane z obecnością *Candida* spp. w przewodzie pokarmowym badanych. Konsumpcja produktów bogatych w nasycone kwasy tłuszczowe korelowała ze zmniejszoną liczebnością drożdżaków w przewodzie pokarmowym. *Candida albicans* wykazywał wrażliwość na wszystkie leki zastosowane w teście, co może zwiększać skuteczność terapii kandydozy.

Słowa kluczowe: *Candida* spp., dieta, próbki kału, antybiotyki, lekooporność

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INTRODUCTION

Candida spp. is the most common member of human gut microbiota and estimated to be present in 40-60% of the general population [1, 2, 14]. It may be present as transient or permanent colonizer in the oral cavity and in the further parts of the gastrointestinal tract. This yeast-like fungus is considered an opportunistic microorganism capable of causing diseases in all parts of the gastrointestinal tract. The most important risk factors associated with *Candida* spp. infections include frequent use of antibiotics, chronic use of alcohol and immunosuppressive drugs, and high-carbohydrate diet [2, 14]. The increased incidence of fungal infections contributes to higher morbidity and mortality rates. This is due to increased antimicrobial resistance and a limited number of antifungal drugs that have many side effects. *Candida* spp. can cause infections of mucous membranes and deep tissues [6, 8, 11].

Although *Candida* spp. strains are mainly responsible for opportunistic fungal infections, they have acquired complex and multifaceted resistance to antifungal treatment. It may be induced in reaction to a compound or due to a genetic mutation resulting from prolonged drug exposure [11]. The rapidly increasing resistance of fungal pathogens to commonly used antifungal drugs is becoming a challenge for modern medicine. Increased drug-resistance is associated with the widespread use of antibiotics. The emergence of resistant strains have contributed to the increased mortality and therefore prompted search for novel antifungals. A thorough drug resistance control should be carried out in the case of treating *Candida* strains exhibiting reduced susceptibility to antifungals [4, 10, 13].

The aim of the study was to demonstrate the relationship between diet and the presence of risk factors associated with the growth of human *Candida* spp. The study was based on the analysis of oral swabs and fecal samples for the presence of *Candida* spp. The results obtained allowed us to indicate the correlation between particular elements of diet and the presence of *Candida* spp.

MATERIAL AND METHODS

Material consisted of oral swabs and fecal samples self-collected by the study participants who had been instructed about the appropriate method for sample collection and storage. Respondents filled in the questionnaire on the frequency of consumption of selected food groups, used drugs, diseases and infections, and the degree of physical activity. The investigation was performed in accordance with the Declaration of Helsinki for Human Research. The research was carried out from statutory funds of Wrocław Medical University No. ST.E090.18.021.

A total of 30 people were examined, including 28 women and 2 men. The study was conducted in Wrocław (Poland) from April to May 2017. The mean age of the study group was 23.8 ± 1.98 years.

The material (feces and oral swabs) was cultured on Sabouraud dextrose agar with actidione (cycloheximide) by GRASO. The significant growth of *Candida* spp. on Sabouraud's medium in the form of white, oval colonies with a characteristic yeast smell was evaluated as a positive result. Then, for selective isolation of yeast, the material was cultured on the chromogenic substrate. The identification of individual species was carried out according to the color of colony growth. *Candida* spp. colonies were classified according to the guidelines of the manufacturer (GRASO Biotech). *Candida albicans* developed green colonies, *Candida krusei* pink, and *Candida glabrata* light purple. Collected samples underwent Gram's staining. Large oval cells arranged in clusters and/or individually indicated the presence of *Candida* spp. The susceptibility of *Candida* to antifungal drugs was determined using the Bio-Rad's FUNGITEST™ Kit, which allows the susceptibility testing of yeasts to six antifungal agents at two different concentrations. The presence of a redox indicator allowed a colorimetric reading. The test included 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, itraconazole and fluconazole.

RESULTS

The study involved 30 people, including 28 women and 2 men. Fecal samples and oral swabs were collected from the participants of the study. Isolates were cultured on Sabouraud's medium. Thirteen strains were obtained after incubation, most were identified as *Candida albicans*, but we also identified *Candida glabrata* and *Candida krusei* (Table 1). All strains demonstrated morphology typical for *Candida* species, and were differentiated on the CHROMagar Candida Medium.

Table 1. Type of strains in the group with positive *Candida* spp. cultures

Isolates	% (n=13)
<i>Candida albicans</i>	84.6
<i>Candida glabrata</i>	7.7
<i>Candida krusei</i>	7.7

In *Candida* spp. positive group, the yeast was reported in the oral cavity, feces and both (the oral cavity and feces). *Candida glabrata* strains were isolated only from feces and *Candida krusei* strains from the oral cavity and feces. We reported a low growth of *Candida* spp. cultures in over 50% of isolates. A significant growth (assessed at 3+) of *Candida* spp. cultures from the oral cavity occurred in two people (Table 2).

Table 2. Characteristic of isolates

Isolation	(n=30) %	Significant growth (3+) %	Moderate growth (2+) %	Low growth (1+) %
Total	43.3	15.4	30.8	53.8
Oral cavity	38.5	100.0	25.0	42.9
Feces	38.5	0	50.0	42.9
Oral cavity and feces	23.1	0	25.0	14.2

Respondents filled in questionnaires that included information on their lifestyle and diet. The questionnaire asked about the use of antibiotics, pregnancy, susceptibility to infections, and previous fungal infections or surgeries. There was no correlation between the incidence of *Candida* spp. risk factors in the study group and the presence of yeasts in the *Candida* spp. positive group.

In the group with positive *Candida* spp. cultures, the majority of respondents declared symptoms such as abdominal pain, flatulence and unrestrained appetite for sweets. The minority of respondents declared restless sleep, nausea and a metallic taste in the mouth. One person did not experience any symptoms listed in the questionnaire (Table 3).

Table 3. Frequency of symptoms in the group with positive *Candida* spp. cultures

Symptoms	%
Abdominal pain	69.2
Flatulence	53.8
Unrestrained appetite for sweets	53.8
Restless sleep	15.4
Nausea	15.4
Metallic taste in the mouth	15.4
Not experienced any symptoms	7.7

Respondents were asked about their diet taking into account particular groups of food products and the frequency of their consumption.

Table 4. Description of population surveyed diet

Specification	% (the whole group, n=30)	% (the group with positive <i>Candida</i> spp. cultures, n=13)	% (the group with negative <i>Candida</i> spp. cultures, n=17)
Elimination diet			
Milk-free	10.0	15.4	5.9
Lactose-free	10.0	7.7	11.8
Gluten-free	13.0	7.7	17.6
Not followed any diet	76.7	76.9	76.5
Frequency of consumption particular groups of food products			
Wheat flour	23.3	15.4	29.4
Wholegrain flour	33.3	15.4	47.1 (p=0.04)
White and wholegrain flour	43.3	69.2	23.5
Natural white quark	66.7	53.9	76.5 (p=0.005)
Yellow cheese	39.9	38.5	41.2
Vegetable oils	70.0	69.2	70.6
Olive oil	20.0	23.1	17.6
Coconut oil	3.3	0	5.9
Butter	3.3	7.7	0
Clarified butter	3.3	0	5.9

Most of respondents did not follow any elimination diet. Respondents could choose several variants of their diets. The most frequently mentioned diets were milk-free, lactose-free and gluten-free. Among the respondents from the group with positive *Candida* spp. cultures, the majority of them had not followed any elimination diet.

In the group with positive *Candida* spp. cultures, more than half declared they ate white and wholegrain flour. In the group with negative *Candida* spp. cultures, healthier wheat flour substitutes were consumed significantly more frequently (p=0.04) than in the group with positive *Candida* spp. cultures.

Over 50% of respondents consumed natural white quark regularly. Average consumption of yellow cheese (eg. Gouda) was comparable in the group with positive and negative *Candida* spp. cultures. Quark were eaten significantly more frequently (p=0.005) in the group with negative *Candida* spp. cultures.

The majority of respondents admitted using different types of vegetable oils to prepare both heated and cold food. Most frequently used were vegetable oils including olive oil. In the group with positive *Candida* spp. cultures, the majority of respondents used vegetable oils, the minority of respondents chose butter. In the group with negative *Candida* spp. cultures the results were comparable.

Frequency of consumption particular groups of food products was presented in Table 4.

The study included 16 *Candida* spp. isolates cultured on Sabouraud medium, including isolates from feces and from the oral cavity. Almost 100% isolates were susceptible to 5-fluorocytosine, amphotericin B,

miconazole, ketoconazole, itraconazole, and fluconazole. A few isolates showed moderate susceptibility mainly to itraconazole. *Candida albicans* was the most susceptible to antifungal drugs. No antifungal resistance was detected in the study group (Table 5).

Table 5. Susceptibility of *Candida* spp. to antifungal drugs

Strains	Isolates from oral cavity (%)	Isolates from feces (%)	Susceptible strains (%)					
			5-fluoro-cytosine	Amphotericin B	Miconazole	Ketoconazole	Itraconazole	Fluko-nazole
<i>Candida albicans</i>	37.5	12.5	100.0	100.0	100.0	100.0	100.0	100.0
	-	12.5	100.0	100.0	100.0	100.0	moderate	100.0
	6.3	12.5	moderate	moderate	moderate	moderate	moderate	moderate
<i>Candida glabrata</i>	-	6.3	100.0	100.0	100.0	100.0	moderate	100.0
<i>Candida krusei</i>	6.3	6.3	100.0	100.0	100.0	100.0	100.0	100.0

DISCUSSION

Diet significantly influences the composition and activity of intestinal microbiota, including fungi. High-fat and high-carbohydrate diets are of particular importance. Specific nutrients change the microbial composition and metabolic activity of the physiological flora [5]. Carbohydrates, proteins and individual fatty acids are most frequently studied compounds that influence the growth of *Candida* in the gastrointestinal tract. Scientific research proves that diet contributes to the growth of human *Candida* spp. [7]. Carbohydrates lead to the highest rated of *Candida* spp. growth in the digestive tract, whereas yeast growth is inhibited by saturated fatty acids [6, 7]. It has been reported that probiotics and flavonoids inhibit *Candida* spp. growth [9, 12].

The influence of diet on the development of *Candida* spp. has been proved by examining fecal samples taken from healthy adults as well as by analyzing their current diets. Hoffmann et al. [7] in 2013 reported a positive correlation between the presence of *Candida* spp. in the human gastrointestinal tract, high carbohydrate intake and a negative correlation between the presence of *Candida* spp. and saturated fatty acids intake. The study described the metabolic activity of *Candida* spp., which is capable of fermenting starch, especially after preliminary amylase digestion in saliva. Therefore, yeast may participate in the decomposition of starch from high carbohydrate products and contribute to the release of simple sugars which are a fermentation substrate for this type of fungus.

In this research the authors reported differences in the consumption of high carbohydrate foods between our respondents. People with positive *Candida* spp. cultures more frequently consumed cereal from purified than from whole-grain flour. The diet of people with negative *Candida* spp. cultures more often included healthier wheat flour substitutes (e.g. rye flour, oat flour, buckwheat flour). Potatoes, which

are a rich source of starch, were more frequently found in the diet of people with *Candida* spp. There were no differences in the frequency of consumption and the type of sweets among the respondents of this study.

Gunsalus et al. [6] evaluated the effect of different types of food fats on gastrointestinal tract infestation by *C. albicans* and compared the role of beef tallow, soybean and coconut oils in the process of *C. albicans* multiplication in the intestines of mice. Beef tallow and soybean oil are rich in long-chain saturated fatty (C16:0 and C18:0) and unsaturated fatty acids (C18:1 and C18:2). Coconut oil is rich in medium- and short-chain fatty acids. Coconut oil used in in vitro conditions inhibited the growth of *C. albicans* and led to the death of strains. This effect was mainly attributed to short-chain fatty acids such as capric (C10:0) and lauric acids (C12:0). On the other hand, long-chain fatty acids contained in soybean oil and beef tallow enhanced the growth of *C. albicans*. The colonization with *C. albicans* was lower in mice fed with coconut oil than in mice fed with beef tallow or soybean oil. The researchers concluded that coconut oil inhibits *C. albicans* growth under in vivo conditions.

In this study, the highest percentage of respondents with positive *Candida* spp. cultures (69.2%) used vegetable oils (such as rapeseed oil or sunflower oil), whereas only 1 person used fat containing short chain fatty acids, such as coconut oil or butter. Comparable results were obtained in the group with negative *Candida* spp., therefore it can be concluded that in the studied material the type of chosen fat had no significant effect on the presence of gastrointestinal *Candida* spp. After analyzing the consumption of products containing a large amount of saturated fatty acids, authors of this research reported that people with negative *Candida* spp. cultures consumed significantly more yellow cheese and quark, which are the source of short-chain fatty acids.

Due to the increasing drug resistance of yeast-like fungi to fungistatic drugs, there have been many reports on the effectiveness of antifungal treatment. Researchers from the Department of Periodontology and Oral Mucosa Diseases at the Medical University of Gdansk (Poland) examined the antifungal resistance profile of isolated yeast-like strains in 197 patients. For this purpose, they used oral isolates obtained from the participants of the study and treated them with 7 fungistatic drugs (nystatin, amphotericin B, fluconazole, miconazole, ketoconazole, itraconazole and flucytosine). The most frequently isolated *Candida albicans* strains (70.4%) showed the highest susceptibility to all antifungals tested. The highest percentage (93.0%) of *C. albicans* strains was susceptible to amphotericin B and nystatin (91.7%). The highest resistance to antifungal agents was observed among *Candida krusei* (2.7%) and *Candida glabrata* (9.9%) strains. *C. krusei* showed 100% resistance to fluconazole and itraconazole. *C. glabrata* strains were most resistant to fluconazole (95.5%). Amphotericin B and nystatin were characterized by the highest probability of clinical efficacy for all isolated yeast-like fungi. Susceptibility to amphotericin was demonstrated in 92.9% of *C. albicans*, 81.8% of *C. glabrata*, and 100% of *C. krusei*. In the case of nystatin, the percentage of susceptible strains was lower and for *C. albicans*, *C. glabrata* and *C. krusei* amounted to 91.7%, 77.3%, and 66.7%, respectively. Azole derivatives were assessed as less effective than amphotericin B and nystatin. 68.3% of *C. albicans* and 13.6% of *C. glabrata* strains showed susceptibility to azole antifungal drugs, whereas *C. krusei* isolates were resistant to all azole drugs tested [3]. Similarly in this study, *C. albicans* strains were most susceptible to the majority of antifungal drugs. 61.5% of strains were susceptible to 5-fluorocytosine, amphotericin B, miconazole, ketoconazole, itraconazole, and fluconazole. 15.4% showed susceptibility to 5 drugs and moderate susceptibility to itraconazole. 23.1% of isolates were moderately susceptible to all the drugs tested. *C. glabrata* isolates were susceptible to all drugs tested except for itraconazole, for which they showed moderate susceptibility. *C. krusei* strains were susceptible to all drugs used in the test.

CONCLUSION

Results of statistical analysis suggests a positive correlation between the consumption of high carbohydrate content foods (such as products made from wheat flour) and the presence of gastrointestinal *Candida* spp. and a negative correlation between the presence of gastrointestinal *Candida* spp. and the consumption of products rich in saturated fatty acids.

Use the Bio-Rad's FUNGITEST™ Kit which allows the susceptibility testing of yeasts to six antifungal agents at two different concentrations determines *Candida albicans* strains are most commonly susceptible to antifungal drugs.

Conflict of interest

The Authors declare no conflict of interest.

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