ACTA FAC MED NAISS

UDC 616.34-002.828

Original article

ACTA FAC MED NAISS 2008; 25 (4): 189-193

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RECURRENT INTESTINAL CANDIDOSIS

SUMMARY

The prevalence of recurrent intestinal candidosis (RIC) has been increasing in recent years.

The aim of the study was to investigate the prevalence of some *Candida* species in etiology of RIC and to examine their antifungal susceptibility.

The study involved 70 patients with RIC. The patients were selected according to criteria that in the previous six months they had minimum two microbiological findings of the same *Candida* species in feces, as well as the symptoms and clinical signs of the digestive tract infection (nausea, disgust, borborygmus, bloating, mushy stool, appearance of mucus in feces). *Candida spp*. were isolated using a standard procedure. *Candida albicans* was (*C. albicans*) identified from other species by the germination test application. Non-albicans species were differentiated using a commercial CandiFast-test (Mycoplasma-International, France).

After using the tests for yeast differentiation, *C. albicans* was found to be the most frequent cause of recurrent intestinal tract candidosis (45 patients - 64.29%). Other species were found in a significantly smaller number of patients (*C. glabrata*-4, *C. crusei*-4, *C. kefyr*-3, *C. parapsilosis*-1, *C. guillermondil*-1, *C. tropicalis*-1, *C. lusiniae*-1). The isolated yeasts showed good susceptibility to amphotericin B, nystatine, 5-fluorocytosine and ketoconazole, while higher percent of isolated species was resistant to econazole, miconazole and fluconazole.

Species *C. albicans* is the most common cause of RIC.

Key words: **recurrent intestinal candidosis, antifungal susceptibility, *Candida spp*.**

INTRODUCTION

Candida species cause superficial mycoses, opportunistic fungal mucosal infections and systemic mycoses (1, 2). Considering the fact that *Candida spp*. are members of physiological flora of human mucosal membranes, there is still a dilemma how to differentiate Candida-infection from Candida-colonization of the intestinal mucosa (1-3). To date, even after numerous researches, the exact phenotype or genotype of Candida spp., which would be responsible for the digestive tract infection, has not been determined (4).

The most severe forms of the digestive tract infection caused by *Candida spp*. can be esophageal and gastrointestinal candidoses arising due to severe primary disease. Esophageal candidosis is mainly the consequence of AIDS or severe immunosuppression occurring due to long-term chemotherapy in the management of leukemia or tumors (1). In

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addition, the patients suffering from leukemia and other hematological malignant diseases are the risk group for development of numerous ulcerations of the gastric mucosa, rarely of duodenal and jejunal mucosa due to fungal infections. Perforations and complications with peritonitis and hematogenic spreading of yeasts into the liver, spleen and lungs are very common in these patients (1). So far, the association between the epigastric complaints and gastrointestinal tract colonization was neither ascertained nor epidemiologically proved. There are data in the literature about delayed healing and the management of gastric ulcer caused by Candida colonization/infection of the gastric mucosa (4, 5).

In the majority of patients, candidosis of the digestive tract can be, under beneficial circumstances, only the consequence of the intestinal mucosa dysbiosis, arising due to primary infection caused by other microorganisms, long-term use of antibiotics, decreased mucosal immunity etc. In such cases, the symptoms are of mild intensity, including increased intestinal peristalsis, bloating, nausea, weak pains, and a number of mushy stools per day. Usually, this condition resolves by administration of antimycotics and an appropriate dietetic regime (1, 4). An adverse outcome means that this coloni-zation/infection recurs in a considerable number of patients. A very important piece of information is that the subjects with the aforesaid symptoms have the findings of the same fungal species in feces and reoccurrence of candidosis despite the applied therapy; there is no long-term history of primary diseases conditioning recurrent fungal infections, such as diabetes, malignant, hematological and endicrinological diseases as well as congenital and acquired immunodeficient conditions. Furthermore, these patients do not receive therapeutical or other treatments which predispose the yeast overgrowth in the digestive tract (1, 4, 6).

Even after numerous epidemiological studies, the risk factors being responsible for repeated Candida colonization/infection of the intestinal mucosa have not yet been determined (4, 7-9). Prolonged use of antibiotics has been a confirmed risk factor for the occurrence of these complaints; however, in the majority of patients with intestinal candidosis, this predisposing treatment has not been documented (4).

The aim of the paper was to investigate the prevalence of certain *Candida* species in etiology of RIC, and to examine their antifungal susceptibility *in vitro*.

MATERIAL AND METHODS

The investigation included 70 patients with RIC. The patients were selected according to criteria that in the previous six months they had minimum two microbiological findings of the same *Candida* species in feces, as well as the symptoms and clinical signs of the digestive tract infection (nausea, disgust, borborygmus, bloating, mushy stool, appearance of mucus in feces). In addition, the findings of neither pathogenic nor conditionally pathogenic microorganisms were reported.

Candida species were isolated by the application of the standard procedure, i.e. by using Sabouraud agar, chromogenic medium (Cromogen albicans, Parquetecnologico de Madrid, Spain) and malt-agar. *Candida spp*. overgrowth was evident after 2-5 days, and they were identified based on macroscopic and microscopic morphological features. *C. albicans* was identified by the application of germination test and chromatogenic medium. Non-*albicans* species were differentiated using a commercial CandiFast test (Mycoplasma-international, France). Also, by using this commercial test, the antifungal susceptibility of the isolated *Candida spp.* strains was determined.

Candifast test is a commercial test providing the investigation of *Candida spp*. susceptibility to amphotericin B, (4 μm/ml), nystatine (200 IU/ml), 5-fluorocytosine (35 μm/ml), econazole (16 μm/ml), ketaconazole (16 μm/ml), miconazole (16 μm/ml) and fluconazole (16 μm/ml).

Based on the manufacturer's instruction for the test use, the isolated strains were marked as susceptible, less susceptible and resistant to the examined antimycotics. By summing up the antimycogram test results, the percent of susceptible strains of certain fungal species in the selected group of patients was determined.

RESULTS

In the patients with recurrent fungal colonization/infection of the digestive tract, the most frequent causative agent was *C. albicans*. Other fungal species of this genus were identified in a smaller number of patients *(Table 1)*.

Table 1. Isolated Candida species from the material of RIC patients

Total number of isolates: *Candida spp*. = 70 strains	
C. albicans	55
C. galbrata	4
C. krusei	4
C. kefyr	3
C. tropicalis	1
C. parapsilosis	1
C. guilliermondii	1
C. lusitaniae	1

All the isolated strains of *Candida spp*. showed good susceptibility to amphotericin B, nystatine, fluorocytosine, ketaconazole (75-100%). The isolated fungi showed the lowest percent of susceptibility to econazole and fluconazole (*C. albicans*-20% of strains susceptible to econazole; 22.22% susceptible to fluconazole; *C. glabrata*-25% susceptible to econazole; *C. krusei* – 25% susceptible to econazole; 25% susceptible to fluconazole); *C. kefyr*-33.33% susceptible to econazole; 33.33% susceptible to fluconazole).

The number of isolates of different Candida species and their susceptibility to the tested antimycotics were presented in *Tables 2* and *3*.

Table 2. Susceptibility to antimycotics of C. albicans and C. glabrata isolated from the material of RIC patients

Antifungals	*C.albicans* / 55 strains	*C.glabrata*/4 strains
	% of susceptible strains	% of susceptible strains
Amphotericin B^{AB} 4µg/ml	100	100
Nystatin ^{Ny} 200 UI/ml	100	100
5-Fluorocytosine^{FC} ^{T} 35µg/ml	100	100
Econazole^{EC} 16µg/ml	20	25
Ketonazole^{KTZ} 16µg/ml	88,89	75
Miconazole^{MCZ} 16µg/ml	33,33	50
Fluconazole^{FCZ} 16µg/ml	22,22	50

Table 3. Susceptibility to antimycotics of C. krusei and C. kefyr isolated from the material of RIC patients

Antifungals	*C. krusei* /4 strains	*C. kefyr*/ 3 strains
	% of susceptible strains	% of susceptible strains
Amphotericin B^{AB} 4µg/ml	100	100
Nystatin ^{Ny} 200 UI/ml	100	100
5-Fluorocytosine^{FCT} 35µg/ml	100	100
Econazole^{EC} 16µg/ml	25	33,33
Ketonazole^{KTZ} 16µg/ml	100	100
Miconazole^{MCZ} 16µg/ml	100	66,67
Fluconazole^{FCZ} 16µg/ml	25	33,33

In the patients with RIC, as a single finding, strains of *C. lusitaniae* and *C. parapsilosis* were detected, and they showed good susceptibility to all the tested antimycotics. In addition, in this group of patients, single findings of *C. guilliermondii* and *C. tropicalis* were reported; they showed good susceptibility to amphotericin B, nystatine, fluorocytosine, and ketaconazole.

DISCUSSION

Candida spp. are members of the normal, physiological flora of the human skin and mucosal membranes. The prevalence of *Candida* colonization of the gastrointestinal tract is extremely high even in healthy, immunocompetent subjects, and ranges from 4% to 88%, depending on the localization in the digestive tract (1, 2). This significant percent of *Candida* colonization of the intestinal mucosa has been found among the healthy population, and is reported in adults and children (10). This is the major reason why it is very difficult to interpret the finding of *Candida spp*. in feces and differ fungal colonization from infection (11).

In the 70's of the past century, a group of authors assumed the attitude that yeast overgrowth on the intestinal mucosa can cause, besides damages of the digestive tract mucosa, neurological and mental disorders and changes, or changes in these patients. These authors introduced the theory of "Candida syndrome" supported by the facts that during yeast overgrowth on the intestinal mucosa, a great amount of gases, alcohol and toxins are produced, which further cause the mucosa damages, allergic reactions which can cause, at systemic level, damages to any part of an organism (4).

The attitudes of the aforementioned authors needed to be officially supported regarding the issue of "Candida syndrome". After considering this hypothesis, the American Academy of Allergy and Immunology in 1986 and the American Medical Association in 1992 brought a general attitude that "Candida Syndrome" was just a supposition which was not brought on the basis of satisfactory and convincing evidence (4).

Since then, a large number of papers has been published, aiming to investigate the pathogenesis of the intestinal tract candidosis from several aspects. In sum, it can be said that after a large number of studies, the majority of authors reject the validity of the problematic syndrome; however, they assume the attitude that yeast overgrowth on the intestinal mucosa can cause the diarrheal syndrome (4, 8, 9).

Our investigation included a group of patients with constant repeating of the diarrheal symptom, followed by positive finding of the same

Candida species in feces. In this group of patients there were no data about possible predisposing diseases, nor possible risk factors, primarily previous antibiotic treatments. All patients were treated by antimycotics (nystatine or ketaconazole) during shorter or longer time intervals. In all of them, the symptoms subsided after the applied therapy, but recurred after 2-4 months.

In this group of patients, as in other studies, the most frequent cause of RIC was *C. albicans* (4, 7, 10, 11). In a considerably lower percent, the causes of RIC were the following non-*albicans* species: *C. glabrata*-4, *C. kruzei*-4, *C. kefyr*-3, *C. lusitaniae*-1, *C. parapsilosis*-1, *C. guilliermondi*-1 and *C. tropicalis*-1). In other investigations related to this topic, the most frequent non-*albicans* species as colonizers of the digestive tract are *C. glabrata*, *C. tropicalis* and *C. guilliermondi* (4, 7, 10, 11). *C. albicans* is a predominant causative agent of fungal opportunistic infections, which is usually explained by its factors of pathogenicity, above all dimorphism, i.e. the ability to turn into a polynuclear form. According to data from the reference literature, this species is the most frequent colonizer, as well as the cause of mucosal infections in humans (1-3).

In the investigation of antimycotic efficacy *in vivo*, there was no antimycotic treatment that could completely eradicate fungi from the intestinal mucosa, i.e. the so-called treatment for "antimycotic decontamination of intestinal mucosa". The applied antimycotic therapy is considered just to decrease the amount of fungi on the intestinal mucosa below the detectible level of 10^2 cfu/ml. Four to five days after the treatment, fungi can be detected again in feces, so that only the absence of symptoms can point to healing. A number of authors emphasize the efficacy of nystatine therapy, both in resolving the symptoms and decreasing the number of fungi below a detectable level (4). By using the commercial antimycogram test, we ascertained a considerably high percent of strains susceptible to nystatine, but also to amphotericin B, 5-fluorocytosine and ketaconazole. Significant percent of strains resistant to econazole, miconazole and fluconazole was reported. Despite the fact that higher prevalence of strains of non-*albicans* species resistant to the effect of azole derivations in vivo was reported, our investigation did not show a significant difference in *C. albicans* susceptibility, compared to non-*albicans* species, to the effects of antimycotics in vitro (12, 13).

Taking into consideration the facts that: a) there is a small number of papers in the reference literature dedicated to the investigation of susceptibility of *Candida spp*. as the cause of intestinal candidosis *in vitro*; b) there are some contradictory findings of antimycotic efficacy *in vivo* and *in vitro* (4, 6, 7); c) a different range of antimycotics in commercial tests is available; d) there has not been a standardized method for a long time for the examination of fungal susceptibility to antimycotics *in vitro*, there was not material enough to compare findings.

In numerous analyses and studies, with the aim to investigate the possible causes and risk factors for the occurrence of fungal colonization/infection of the digestive tract, controversial data have been obtained, so that etiopathogenesis of the digestive tract mucosa remains still unclear (11). Epidemiological studies have not ascertained that dietetic regime, food additives, use of oral contraceptives can affect the occurrence of digestive tract candidosis; therefore, it is supposed that they do not affect its recurrent form. The current opinion is that digestive tract candidosis arise due to decreased nonspecific mucosa immunity. In the past, this clinical entity used to be associated with newborns, small children and elderly population. Today, these infections stand for a more serious medical problem, both for considerable incidence and highly frequent development of the recurrent chronic form. This practical problem has not been clarified yet, though after numerous studies dedicated to the investigation of the recurrent form of genital candidosis, it can be only assumed that dysregulation of the local immune response occurs on the mucosa, due to hyperreactivity type I, i.e. Th-2 local cellular reactivity, or due to impaired immunotolerance, which is usually present on mucosas (10, 11).

Based on the obtained results in this study, it can be concluded that the most frequent cause of the recurrent form of fungal colonization/infection of the intestinal mucosa is *C. albicans*. In addition, the investigation showed that the most efficient antimycotics for the isolated strains of *Candida spp. in vitro* were amphotericin B, nystatine, 5-fluorocytosine and ketoconazole.

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REKURENTNA INTESTINALNA KANDIDOZA

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SAŽETAK

Prevalencija rekurentne intestinalne kandidoze (RIK) u porastu je poslednjih godina.

Cilj rada bio je ispitati zastupljenost pojedinih vrsta gljiva roda Candida u nastanku rekurentnog oblika gljivične infekcije intestinalnog trakta i utvrditi njihovu osetljivost na antimikotike u uslovima in vitro.

Istraživanjem je obuhvaćeno 70 bolesnika sa RIK. Bolesnici su odabrani po kriterijumu da su u proteklih 6 meseci minimum dva puta imali mikrobiološki nalaz iste vrste gljiva roda Candida u fecesu i simptome i kliničke znakove infekcije digestivnog trakta (muka, gađenje, pretakanje, nadimanje, kašaste stolice, veći broj stolica u toku dana i pojava sluzi u stolici). Gljive roda Candida izolovane su primenom standardne procedure. Vrsta Candida albicans (C. albicans) identifikovana je primenom testa germinacije i primenom hromatogenog medijuma (Cromogen albicans, Parquetecnologico de Madrid, Spain). Ne-albicans vrste diferencirane su primenom komercijalnog CandiFast-testa (Mycoplasma-international, France).

Korišćenjem testova za diferenciranje gljiva, vrsta C. albicans utvrđena je kao najčešći (45 pacijenata-64,29%) uzročnik rekurentnog oblika gljivične infekcije intestinalnog trakta. Ostale vrste identifikovane su kod značajno manjeg broja bolesnika (C. glabrata-4, C. krusei-4, C. kefyr-3, C. parapsilosis-1, C. guilliermondii-1, C. tropicalis-1, C. lusitaniae-1). Korišćenjem komercijalnog antimikogram testa, kao efikasni antimikotici utvrđeni su amfotericin B, nistatin, fluorocitozin i ketokonazol, ali je detektovan visok procenat rezistentnih sojeva prema dejstvu ekonazola, mikonazola i flukonazola.

C. albicans je značajno češći uzročnik RIK u odnosu na ne-albicans vrste gljiva roda Candida.

Ključne reči: **Rekurentna intestinalna kandidoza, Candida spp., osetljivost na antimikotike**