CONDITIONALLY PATHOGENIC FUNGI
IN RECREATIONAL WATERS*

ABSTRACT: The improvement of health and life conditions depends on various environmental factors. The exposition to organic and inorganic pollutants, as well as to the broad specter of microorganisms is one of these factors. Medically important fungi have been increasing their number recently, especially in urban and in recreational zones. Some of them, first of all molds and yeasts, are involved by different means in causing more or less serious deseases of man and animals. Frequency of alergic symptomes and human mycotic lesions increased significantly during last decades. Such phenomena have provoked more scientific attention recently.

According to the available literature data, micro-fungi, causing mycoses and „environmental” fungi too, could be considered as an important factor of health risk, being neglected and underestimated so far, especially in analyses of safe use of recreational waters and surrounding areas, among them swimming pools, river and sea beaches. On the basis of such statement there arises conclusion that water and ground of recreational zones could serve as vectors in transmission pathways of potentially or conditionally pathogenic fungi, being dangerous especially for immunocompromised individuals, which suggests inclusion of qualitative and quantitative composition of fungal community into a continual monitoring of hygienic status of recreational zones.

KEY WORDS: Fungi, recreational waters, health risk, mycoses, monitoring

INTRODUCTION

During the XXI century, the significant increase of incidence of infections caused by so-called „environmental” fungi has been anticipated and the fact is that ecological microbiologists are more familiar with this group of fungi than clinical ones, especially not acquainted with them to the extent which the seriousness of this problem deserves (Koontz, 1998).

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The incorrect opinions considering human and mammal parasitic fungi are still widely distributed, especially regarding the thought that relatively small number of fungi parasitize on Vertebrates and that these so-called „medicinal fungi” represent special group of Mycota whose confusing nomenclature and taxonomy additionally complicate their study. Nevertheless, before discussing such attitudes, it would be necessary to remind oneself that in everyday terminology use one should distinguish between the term „parasitic” and „pathogenic”, where as a pathogenic the parasite who could cause a disease of the host species is characterised? Also, one can speculate that every parasite in certain circumstances and under certain conditions could become pathogenic? Recent clinical position of synonymizing these two terms have been formed due to the fact that the majority of parasites could cause more or less serious diseases.

Most of our knowledge about virulent determinants of pathogenic and potentially pathogenic fungi comes from an infected host (person), mainly from experimental animal models and recently from „in vitro” studies of cell cultures (M e n d e s - G i a n n i n i, 2000). Fungi represent intra and/or extracellular parasites, and parasite phenomenon depends on complementary surface molecules. It has been characterised as event of cohabitation where fungi, creating stable conditions for their survival, recognize specific tissue of host as an attractant. Infection in most cases appears with mechanic injuries and unadequate dressed wounds which become secondarily infected by microorganisms that are present in water.

It is also important to remind ourself that besides parasitic, fungi might cause health disorder in humans in some other ways. If they are ingested, they might act like poison and in contact with, in some way sensitive persons, they might cause allergic reaction (A i n s w o r t h, 1968).

Inoculation of infective agents is possible superficially, by dermic way (penetrated injuries, lacerations, being pricked when swimming); by ingestion or inhalation. Very few data considering the quantity of absorbed water during „typical” exposition to recreational waters are avaliable. The most often used standard is 100 ml per day (H a a s, 1983). Depending on the depth of inoculation and the sort of microorganisms that are inoculated, serious infections of tissues are possible: deformities and loss of function and even systematic infections. Piodermiae, eye and ear infections, as well as urogenital tract infections, are possible even without previous mechanic injury, especially in the parts where wastewater has been mixed with water that is used for recreational activities.

Certain kinds of organic dust when being inhalated do not cause visible damage, and others cause clinic symptoms in three ways:

1. Acting as allergens (complete antigens) cause sensibility and cause alergic alveolitis (especially in case of uneffective alveolar macrofag);
2. Cause direct toxic or mechanic irritations according to the type of „irritation by strange body”;
3. Directly cause lung infection (M i k o v, 1995).

Inhaled dust or fungal spores, not bigger than 5 mm in diameter, are kept on mucous membrane of upper respiratoric parts, while particles smaller
than 5 \mu m penetrate the lower respiratoric parts (respiratoric bronchialae and
alveolae). For diagnosis it is important to prove the connection of time and
space with the source of infection. The members of genus *Aspergillus* belong
to the most frequent contaminants. The illness caused by *Aspergillus* species is
characterised by inflammatoric granulomatous lesions. Allergic form of lung
aspergillosis appears in predisposed atopic-astmatic persons. These persons spit
out the spittle that consists of eozinophylls, micelium of fungi, and in blood
increased eozinofilia can be detected. Illness gives positive reactions on skin
and precipitin against *Aspergillus fumigatus*.

Hypersensitive pneumonitis (allergic alveolitis) is lung illness which is in
most cases caused by inhalated thermophyllic actinomycets and fungi. M o -
reno — A n c i l l o et al. (1997) report that they have noticed favourable
conditions for the growth of fungi in closed warm swimming pools that were
visited by their patients. To determinate possible etiologic agents, cultures
from several parts of the swimming pool have been isolated. These isolates ha-
ve shown significant growth of thermophyllic mycobiota where *Neurospora,
Aspergillus* and *Pullularia* species have been isolated from samples.

Development of quality of life and health of people is complex function
of different conditions of living environment. Here, among others, exposure to
organic and inorganic pollutants as well as to the wide spectrum of microorga-
nisms belongs too. The frequency of presence of medically important fungi
has increased in urban and recreative zones. Some fungi, first of all yeasts and
molds, are included by different ways in causing pathologic alterations in hu-
man and animal organisms. Allergenic symptoms and human mycotic lesions
as onychomycosis for example, have been increasing in the last decade (M e n-
d e s et al., 2000). The same authors the increasing presence of potentially path-
genic fungi on the beaches of Portugal explain as the consequence of increa-
sed quantities of organic waste-materials of anthropogenic origin.

Fungal infections on humans and animals, so called mycoses, may be
classified according to the part of the body on which they parasitize, to „cuta-
neous”, „subcutaneous”, „deep” or „systemic” ones, etc. This kind of classifi-
cation, mainly based on medical mycologic tests make it easier to experts to
come to realiable diagnosis. However, this kind of classification is not correla-
ted with the types of parasitic fungi, especially from taxonomic point of view.

Among endogenous mycoses, mainly candidiases are involved (caused by
*Candida albicans*), while exogenous mycoses involve coccidiomycosis (caused
by fungus *Coccidioides immitis*), adiaspiromycosis (*Emmonsia* species), histop-
plasmosis (*Histoplasma capsulatum*, imperfect form of *Gymnoascus demon-
breanii* and other systemic mycoses) the cause of which might be *Cryptococ-
cus neoformans* (cryptococosis), *Aspergillus fumigatus* and others (aspergillo-
sis), *Sporothrix schenckii* (sporotrixosis), etc. (A i n s w o r t h, 1968).

Since fungi may cause human and animal illnesses, they are the subject
of studies of both mycology and medicine (pathology). As parasites on hu-
mans and animals, fungi may attack skin and keratinised tissues: nails, fur, ha-
ir, but also inner organs (organs for digestion, lungs, brain and so on). In the
first case they are superficial parasites, very often opportunistic ones, with cer-
tain localisation of pathologic processes that they cause. In other case fungi
may cause systemic or deep mycoses, apscessee in different organs, leukemia, endocarditis and other illnesses. Clinical symptoms vary very much and range from small surface nodullae on hair and skin escharification on which spores of the fungus may be found, as well as deep inner illnesses which may have fatal result (M u n t a ñ o l a - C v e t k o v i ć, 1987).

Human and animal mycoses are rare and less dangerous than those on plants. They mostly appear on weakened and immunocompromised persons, so they could be considered as opportunistic infective agents.

To be potentially human pathogenic organisms, fungi do not have to be direct infective agents. They may cause allergic and hypersensitive reactions. Spores that in smaller and higher concentrations hang in the air, especially in the dust of contaminated areas, are important as „inhalation” allergens. Members of many families of fungi especially of the Aspergillus, Penicillium, Alternaria, Cladosporium, and Mucor genera, in their spores contain substances that can cause irritative syndroms or sensibilization.

Prerequisite of pathogenesis of many systemic invasion of fungi on humans and animals is incorporation of fungi into phagocytes. This way the fungus provides the habitat, where occurs in vivo transformation of mycelial into single-cell (yeast-like) round forms, the fungal answer to the defensive mechanisms of a host organism and at the same time it is the first step of penetration of fungus into cells and tissue of a host organism. This kind of fast morphological adaptation to intracellular habitat is characteristic for human pathogens such as: Histoplasma capsulatum, Cryptococcus neoformans, Coccidioides immitis and others.

Hyphae of some pathogenic members of Zygomycotina and of the genus Aspergillus keep the mycelial form when penetrate into host tissue, using specific ways of adaptation to the new environment. Inhalated spores of Aspergillus fumigatus after phagocytosis germinate into short, wide and bronched hyphae (ø 5—10 mm, unlike the normal of ø 3—5 mm), known as actinomyctoid that may be found with chronic aspergillosis. In infections caused by the members of Mucor and Rhizopus genera it can be noticed the increase of volume and thickness of inner wall of hyphae as an attempt of adaptation to environmental antagonism. In the last stadia of some animal illnesses hyphae recover normal vegetative mycelial form which testify about overcome resistance of the host tissue (M u n t a ñ o l a - C v e t k o v i ć, 1987).

LITERATURE REVIEW

Besides other microorganisms from the Mediterranean beaches and surfaces of swimming pools, fungal species have been isolated, too. There are a great number of micro-fungi, potential or conditional pathogens that may be contacted via beach sand, but there is no epidemiological evidence about transmission of pathogens by this way (EOS/DRAFT/98.14, 1998, WHO, 2000).

The widespread use of sea- and other natural surface waters for recreational purposes in recent years has brought to the question a problem of exposure to potential risk of health of bathers, swimmers and people who do some other
ways of recreation on water. Bacterial indicators are widely used in estimation of presence of potential pathogen in water for recreation and in the soil as a part of recreational zones. However, bacterial indicators, especially faecal coliforms, indicate only indirectly the possible presence of some potentially pathogenic fungi, first of all the presence of some kinds of yeasts. On the basis of investigation of 1576 samples from six recreational beaches in Israel, Shemesh et al. (2000) have concluded that only 4.5% of samples contained faecal coliforms in quantities not allowed by the regulative. Yeasts and molds were present in great number (91%) of samples. From 44 identified species, 15% belonged to the genus of Rhodotorula, 12.5% to Candida humicola and 12.3% to the Candida albicans species. Most of mold isolates belonged to the Aspergillus and Penicillium genera. Since among members of the Candida and Aspergillus genera exist species that are considered to be conditionally pathogenic, these authors recommend obligatory inclusion of „mycotic” parameters as additional indicators in estimation of relative safe use of recreational waters.

The similar results published Arvanitidou et al. (2000) who, investigating 197 samples of sea water during the summer season, found 100% samples contaminated by particles of filamentous fungi, while 15% were contaminated by yeasts. Mycelial fungi belonged to the genera: Penicillium (isolated from 135 samples), Aspergillus (isolated from 113 samples) and Alternaria (found in 47 samples), while yeast-like Candida species have been found in 8 samples. The number of yeasts was found to be in significant correlation with the number of total coliform bacteria. Between the filamentous fungi quantity and number of bacterial indicators of organic pollution, such a correlation has not been found. On the basis of these facts the authors have concluded that the sea water should be considered as a potential vector in transmission ways of conditionally pathogenic fungi, especially for immunodeficient persons, consequently recommending the obligatory continuous monitoring from this point of view of sanitary conditions of recreational zones.

In the frame of epidemiologic investigations done on two beaches in the areas of Malaga in Spain, it has been found that indicators of faecal pollution show highly significant coefficient of regression especially with the presence of dermatomycotic fungi. Only a number of Escherichia coli was found to be in positive correlation with the number of Candida albicans. On other beaches the quantity of faecal Streptococae has also been found to be in high positive correlation with dermatomycotic population. Again coliforms have been found to be in the most obvious correlation with Candida albicans (Borrego et al. 1991).

The investigations of the beaches of Portugal seashore (Sous, 1990) revealed the presence of dermatomycota (here the traditional inadequate term — „dermatophyta” has been put out of usage since fungi are not plants and suffix -phyta would wrongly direct to their belonging to the plant kingdom) in 42% analysed samples of sand beaches. Most frequently present dermatomycota were: Trichophyton mentagrophytes, Trichophyton rubrum and Microsporum nanum, all of them isolated from sand areas heavily loaded by organic waste. Saprobic (not „saprophytic”) fungi such as Aspergillus candidus, Asper-
*Gillus ochraceus* and *Aspergillus fumigatus*, have been isolated mostly from flooded and tidal areas (Izquierdo et al., 1986).

*Candida albicans* and other members of the family *Candida* have been isolated from the samples of soil of sand beaches in the south of France (Bernard et al., 1988). The same investigations have confirmed the presence of 8 keratinophilic and 11 nonkeratinophilic species of potentially pathogenic fungi. Izquierdo et al. (1986) have isolated 16 species from sand from the beach of north Spanish Mediterranean seashore, some of them belonging to the potentially pathogenic fungi. Most of the isolates belonged to the *Penicillium, Aspergillus* and *Cladosporium* genera.

Ghinsberg et al. (1994) have isolated fungi from all samples of sand from beaches, but not from sea water at the same localities. Boiron et al. (1983) have studied all kinds of fungi in the sea water and sand from the beach in the same area and come to the conclusion about similar qualitative composition of bacteria in the sand and sea water. They recorded the absence of *Candida albicans* and presence of yeasts exclusively of the marine origin.

The isolated fungi belonged to: *Candida tropicalis, Candida parapsilosis, Candida langeronii, Candida guilliermondii, Trichosporon cutaneum* and *Torrulopsis* sp. The most frequent species found in sand of sea beaches in another study of Spanish seashore belonged to the genera: *Penicillium, Aspergillus, Cladosporium, Alternaria, Mucor, Monilia, Cephalosporium, Verticillium* and *Chrysosporium* (Rosés Còdina et al., 1988). The absence or low incidence of *Candida albicans* species have been reported by other authors too (Rosés Còdina et al., 1988; Figueras et al., 1992).

Quantitive structure of fungal population in 180 samples collected from 42 Spanish Mediterranean beaches ranged to the several hundred thousand cfu/g (colony forming units). The most frequently isolated species belonged to the genera of *Penicillium, Cladosporium, Aspergillus, Acremonium, Alternaria* and *Fusarium* (Larrondo and Calvo, 1989). The results of investigations performed in the region of Atica in Greece, revealed the following qualitative composition of fungi: *Candida albicans, C. crusei, C. tropicalis, C. guilliermondii, C. rugosa, Pitirosporum orbiculare, Fusarium sp., Penicillium spp., Mucor sp., Helminthosporium sp.* and *Aspergillus niger* (Papadakis et al., 1997). *Candida albicans* and other species from the same genus, as well as species from the genus *Fusarium* and the species *Pitirosporum orbiculare*, represented pathogenic or potentially pathogenic community. Mendes et al., (2000) investigating 42 Portugal beaches, flooded, not flooded, intermedial, and area flooded only during high tide find frequent presence of filamentous fungi from the *Penicillium, Aspergillus, Acremonium, Fusarium, Cladosporium* and *Rhizopus* genera, on all investigated beaches and in all three studied areas. Yeast-like fungi, such as: *Candida, Scopulariopsis, Trichophyton* and *Cryosporium*, were found on only few beaches, in more frequent flooded zones and with the frequent presence of people, especially in July and August. Qualitative and quantitative composition of fungi and characteristics of distribution were similar in all studied beaches. The authors conclude that fungi are good indicators of pollution of beaches by organic waste from the consumers of the-
se recreational zones, as well as by the waste brought by the tidal wave in recreational zones.

Boiron et al. (1983) when examining yeast of medical importance, in sand and in sea water of „Sainte-Anne“ beach in Guadalupa, isolated yeast belonging to the genera: Candida, Torulopsis and Trichosporon. Buchalo et al. (1998) isolated three species of filamentous fungi from the surface water of the Dead sea: Gymnascella marismortui (Ascomycotina), which is described as a new species, Ulocladium chlamydosporum and Penicillium westlingii (Deuteromycotina). The isolated cultures could not grow on agar without salt, which suggests the adaptation of fungi to the hyper-saline conditions in that sea.

In vitro studies of human pathogenic fungi from samples from the beaches of Hawaii have been done by Anderson (1979). On this occasion, 4 pathogenic fungi are isolated: Candida albicans, Trichosporon cutaneum, Microsporum gypseum and Trichophyton mentagrophytes. During experimental simulations of conditions that exist on beaches, all species survived for six months, and it shows that they can be the source of infection during a significantly long period of time. Watering and drying sand alternatively caused the shorter period of surviving of all species except Microsporum gypseum. The increasing of temperature resulted in general shorter period of survival; 45°C was inhibitory temperature, with exception of Trichosporon cutaneum, which survived that temperature level for almost 6 months. The level of salt did not influence the survival of this fungus.

Microbiological community of 108 samples of water of 6 swimming pools and 3 lakes with beaches has been examined to evaluate the role of recreational waters as possible source of human illnesses in the areas of Araraquara in Brazil (Falcão et al., 1993). In this study Candida albicans, yeasts and other dermatomycota were isolated. This study has shown that recreational waters used by the population of Araraquara can be contaminated with potentially pathogenic microorganisms and could be the source of infection.

When watching 4 recreational beaches on the lake Ontario, Sherry et al. (1979) have come to the conclusion that opportunistic pathogen Candida albicans appears in water near beaches due to the faecal contamination of water. The highest number has been recorded during July and August, what was in positive correlation with the highest number of consumers of recreational zones in the course of these months.

Epidermophyton floccosum and species belonging to the genus Trichophyton cause superficial fungal infections of hair, nails and skin. Infection of the skin of feet, mostly among toes, so called Tinea pedis (Aho & Hirn, 1981) is characterised by symptoms that involve ulceration, holes and cuts of skin with strong scab. Tinea pedis can be transferred by direct contacts, and in swimming pools it usually happens by physical contact with surfaces such as floors in showers and dressing rooms that have been contaminated with infective fragments of skin (PWTAG, 1999). This fungus colonizes stratum corneum where optimal environmental conditions for this species exist. In vitro experiment shows that it is necessary approximately 3—4 hours for fungi to initiate the infection. This infection often happens among beach rescue squad and sports swimmers and it is considered as a relatively benign. The only so-
Source of dermatomycota in swimming pools and baths are the swimmers who are infected, so in monitoring of this kind of fungal infection and in the control of expanding of this disease, education is very important. Disinfections of swimmers’ feet, wearing sandals in showers and dressing rooms as well as regular disinfections of floors and bottom of the pool could reduce the infection. People with „athletic feet” and with similar dermal infections should not use public swimming pools or baths (Al-Doo r y & Ramsey, 1987; Public Health Laboratory Service Spa Pools Working Group, 1994).

The investigations of micro-fungi in recreational and potentially recreational waters in our country are relatively new and rare. Maria Muntañola-Cvetković and Bosiljka Ristanović, (1977, 1980) should be considered as founders of these investigations in Serbia. They studied population of micro-fungi of South Adriatic and come to conclusion about the link of anthropogenous factor and qualitative and quantitative composition of fungal community and about the importance of some species originating from the coastal soil. Some works deal with alochthonous micro-fungi in the lakes like the Savsko jezero (Ljajlević, 2000), Vlasinsko jezero (Vukojević et al., 1997) and water reservoirs Grošnica and Gruža (Ranković, 1998). As a rule, in all reports the presence of Aspergillus, Penicillium, Alternaria, Cladosporium, Trichoderma, Mucor and Rhizopus species, and other potential allergenic and fungal conditional causes of infection with immunodeficient persons, are noticed.

CONCLUSION

According to the available literature data, micro-fungi, causing mycoses and „environmental” fungi too, could be considered as important factors of health risk, being neglected and underestimated so far, especially in analyses of safe use of recreational waters and surrounding areas, among them swimming pools, river and sea beaches. On the basis of such statement there arises conclusion that water and ground of recreational zones could serve as vectors in transmission pathways of potentially or conditionally pathogenic fungi, being dangerous especially for immunocompromised individuals, which suggests the inclusion of qualitative and quantitative composition of fungal community into a continual monitoring of hygienic status of recreational zones.

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УСЛОВНО ПАТОГЕНЕ ГЉИВЕ У РЕКРЕАЦИОНИМ ВОДАМА

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Резиме

Флукпација присутности рода и врста гљива од медицинског интереса и значаја порасла је у последње време у нашем природном окружењу, а нарочито у урбаним и рекреационим зонама. Неке гљиве, пре свега кваши и пlesen, укључене су на различите начине у изазване патолошких стања код људи и животиња. Аллергенски симптоми и хумане микотичне лезије у значајном су порасту у последњој десети. Ове појаве проузроковали су већу пажњу научне јавности последњих година, посебно из разлога што се данас схвата да је унапређење квалитета живота и здравља код људи комплексна функција различитих услова животне средине. Ту, између осталих, спада и излагање органским и неорганским пољутантима и широком спектру микроорганизама.
Преглед доступних података из литературе омогућава закључак о микрогљивима као факторима ризика, који су досад били занемаривани у анализама здравствене безбедности коришћења рекреационих вода, поготову базена и речних и морских плажа. На основу оваквих налаза закључује се да вода и тле рекреационих зона треба да се посматрају као потенцијални вектори у трансмисионим путевима (потенцијално) патогених гљива, посебно за имунокомпромитоване особе. То подвлачи потребу укључења квалитативног и квантитативног састава микрогљива у континуираном мониторингу хигијенске исправности рекреационих зона.