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Original Research

Hirudo medicinalis Linnaeus, 1758 – a Probable Vector of Transmission of Fungi Potentially Pathogenic for Humans; Initial Studies

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Abstract

A high incidence of ample diseases with a complicated etiology, including mycoses of various origins, determine the implementation of unconventional methods, including hirudotherapy, to their treatment.

The study was aimed at assessing the mycological purity of medical leech body covers, their jaws and gastrointestinal systems – oesophagus, middle and posterior intestines, and the purity of water from an aquarium they were kept in.

The experimental material were 20 European medical leeches (*Hirudo medicinalis*) and water originating from their breeding. Fungi were obtained according to our own procedures and mycological diagnostics was conducted following standard procedures applied in mycological laboratories.

In total, 22 species of fungi belonging to 13 genera were isolated. The material studied was found to contain fungi classified as potential pathogens: *Candida albicans, Candida guilliermondii, Candida krusei* and *Candida tropicalis*, as well as numerous saprotrophs with a decreased pathogenicity potential.

In view of the results obtained in this study, and a prospective application of hirudotherapy, a question may arise as to whether sterility of cultures and health status of leeches are sufficient and safe for patients. Isolation of a relatively high number of fungi considered pathogenic from leeches poses a potential hazard for people who could have contact with non-sterile therapeutic material, which in this case leeches are.

Keywords: leeches, people, vector of broadcast, yeast-like fungi

Introduction

A high incidence of ample diseases with complicated etiology, including mycoses of various origin, results in a situation where physicians increasingly often opt for unconventional treatment methods, e.g. hirudotherapy. As a nursing-therapeutic procedure it should be absolutely safe. It is determined by the quality of leeches applied, the source of their origin, and culture and transport conditions. Various microorganisms, including those that are pathogenic and potentially pathogenic, accompany leeches living in various open water reservoirs. They may infect the body of a leech and be transferred to the blood of future hosts, thereby also to patients. Then, they may become a causative agent of such diseases as cellulitis, eye infections, arthritis, myocarditis, peritonitis, meningitis and sepsis. For this reason, only leeches cultured under isolated laboratory conditions without contact with the external environment and fed in a controlled way are chosen for hirudotherapy. In this way, they should assure full safety to patients treated with them. Another case in point is how important hirudotherapy

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is in unconventional medicine. Is quality, understood as purity and sterility of the leeches used, good enough to use them for therapeutic purposes? That is why the objective of the reported study was mycological assessment of the purity of body covers of medical leeches, their jaws and other parts of their gastrointestinal system – oesophagus, middle and posterior intestines, and purity of water from the aquarium the leeches used in the study were kept in.

Materials and Methods

The experimental material consisted of 20 medical leeches (*Hirudo medicinalis*) and water from their culture. The leeches were received as a gift from Andrzej Cios, who bought them at the BIO-GEN company (http://www.biogen.pl/docs/ulotka%pijawki.pdf).

Each leech was initially washed alive in Sabouraud's broth to rinse potential fungi out of the cover-muscular sack. Cultures were incubated at 37ºC for 48-72h. The cultures were incubated at 37ºC, corresponding to the body temperature of man with a lowered redox potential, i.e. temperature typical of infected and damaged tissue. Next, the jaws, oesophagus and posterior intestine of three leeches selected at random were prepared. The jaws, posterior intestine and oesophagus were incubated in Sabouraud's broth at 37ºC for 48-72h. Afterwards, the entire material was poured on solid Sabouraud's medium and incubated again at 37ºC for 48-72h.

A membraneous filtration method was applied for the isolation and determination of the number of fungal colonies present in the aquarium water. A water sample (250 ml) was passed through a Millipore membrane filter with pores of size 0.45 μm using a vacuum pump PL 2/2 (0.095 MPa) at a pressure of 0.2 bar. Next, the filter was placed in a beaker with 20 ml of 0.9% sterile NaCl in order to reduce the potential number of spores of mould fungi, and shaken for 0.5 h. Next, a sterile pipette was used to sample 1 ml of the resultant suspension and inoculate it on a Petri dish with solid Sabouraud's medium. The filter after shaking was also placed on a Petri dish with solid Sabouraud's medium. The cultures were incubated for 48-72 h at 37ºC and subjected to a macroscopic examination.

Afterwards, colonies of fungi obtained were counted, screened to Sabouraud's slopes with chloramphenicol and incubated again at 37ºC for 48-72h. The macroscopic assessment was performed on the basis of characteristics of grown colonies, while the microscopic characteristics of fungi in live preparations in a drop of water stained with methylene blue and in micro-cultures incubated for 48-72-144h [1] at 37°C on Nickerson's medium. The microculture run on Nickerson's medium enables identifying yeast-like fungi occurring in one isolate. The use of Nickerson medium is extremely legitimate with attention to the frequency of isolation of multispecies strains. Mycological diagnostics was conducted using morphological and biochemical characteristics of the isolated fungi (zymograms, auxanograms) and confirmed using chromogenous medium (ChromAgar Candida).

Fungi were identified on the basis of keys by: de Hoog et al. [2], Howard [3], Kurtzmann, Fell [4], Lodder, Kreger-van Rij [5], and Midgley et al. [6]. Photographic documentation was made and the fungi were catalogued in the register of species forming the collection of the Chair of Mycology.

Results

Analyses conducted in the study demonstrated the presence of yeast-like fungi in all samples from the cover-muscle sack, the jaws and samples of water. The fungi grew in the form of a film or a sediment at the bottom of the tube. No fungi were recorded in the oesophagus nor in the posterior intestine (Table 1).

In total, 22 species of fungi from 13 genera were isolated in the study.

The most frequently isolated fungi were: *Debaryomyces polymorphus, Debaryomyces hansenii* and *Pichia guilliermondii.* In turn, *Debaryomyces mellissophilus, Kluyveromyces polysporus, Metschnikowia pulcherrima* and *Trichonosporoides spathulata* were recov-

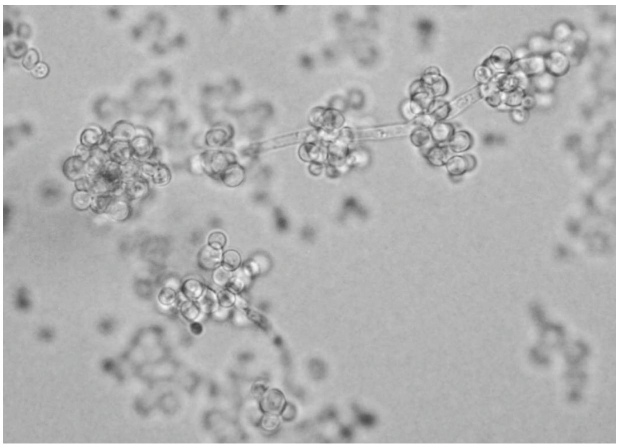

Fig. 1. *Candida albicans* - microculture on the Nickerson agar.

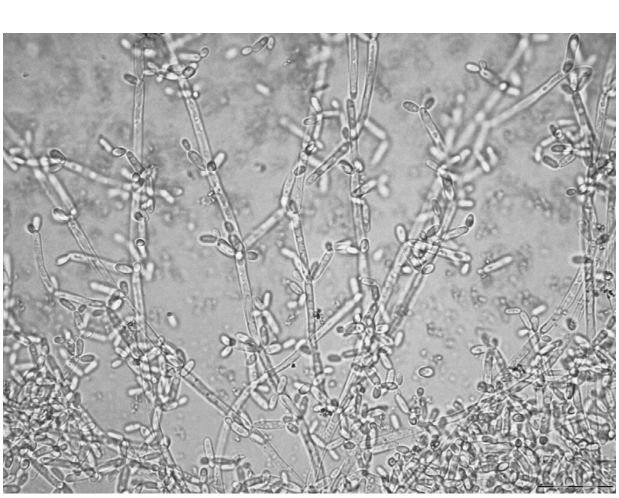

Fig. 2. *Candida krusei* - microculture on the Nickerson agar.

Table 1. Simultaneous presence of yeast-like fungi species in one isolate from macroculture.

No	List of species	Place of isolation	One-in-one isolate	Two-in-one isolate	Three-in-one isolate	Four-in-one isolate	Five-in-one isolate
1	*Candida albicans*	aqw, jaw	x				
2	*Candida guilliermondii*	jaw, cms	x	•		•	
3	*Candida krusei*	jaw, cms	x	•			
4	*Candida tropicalis*	aqw jaw	x				
5	*Debaryomyces castellii*	cms	x				
6	*Debaryomyces hansenii*	cms	x	•			
7	*Debaryomyces mellissophilus*	cms		•		•	
8	*Debaryomyces polymorphus*	cms	x		•	•	
9	*Debaryomyces pseudopolymorphus*	cms	x				
10	*Kluyveromyces polysporus*	cms			•		
11	*Lipomyces starkeyi*	aqw, cms				•	
12	*Metschnikowia pulcherrima*	cms		•	•	•	
14	*Pichia guilliermondii*	cms					•
15	*Rhodosporidium malvinellum*	cms		• •		•	
16	*Stephanoascus farinosus*	cms				•	
17	*Trichosporon ashii*	aqw	x				
18	*Trichosporon asteroides*	aqw	x				
19	*Trichosporonoides oedocephalis*	cms					•
20	*Trichosporonoides spathulata*	cms		•			
21	*Yarrowia lipolytica*	cms					•
22	*Oosporidium sp.*	cms					•
23	*Schizosaccharomyces sp.*	cms				•	•

Legend: •———• more than one species of yeast-like fungi in one macroculture,
aqw – aquarium water, cms – cover – muscular sack, jaw – jaws.

ered less frequently, whereas *Candida albicans, Candida krusei, Candida tropicalis, Debaryomyces castellii, Debaryomyces pseudopolymorphus, Rhodosporidium malvinellum, Schizosaccharomyces, Stephanoascus farinosus, Trichosporon ashii, Trichosporon asteroides* and *Yarrowia lipolytica* occurred occasionally. In a few cases simultaneous presence of a few fungal species in one test sample was found (Table 1). In the water the leeches were cultured in, analyses confirmed the presence of the following fungi: *Candida albicans, Candida tropicalis, Lipomyces starkeyi, Trichosporon ashii* and *Trichosporon asteroides*. In addition, the test material was found to contain fungi classified as potential pathogens: *Candida albicans* – Fig. 1, (BSL-2), *Candida guilliermondii* (BSL-1), *Candida krusei* – Fig. 2, (BSL-2) and *Candida tropicalis* (BSL-2), as well as numerous saprotrophs with a lowered potential of pathogenicity (Table 2).

Discussion

The medical leech *Hirudo medicinalis* has been known in unconventional medicine since the 18th century. The earliest information on methods of using leeches for medical purposes originates from the late Hellenic period and was written by a Greek, Nikandros, who described how "blood-sucking worms are applied to those places of the body that should be released from stasis of blood and juices." In later ages, medical leeches were also used extensively. The peak period in the use of the medical leech for treatment came in the 18th and 19th centuries (during that period, the annual number of *H. medicinalis* used for therapeutic purposes in France amounted to some 80 million, and the incomes from sales of leeches were higher than those from sales of cereals). Leeches lost importance only in the 20th century [7]. Recent years, however, have seen a return of this species to

Table 2. Specification of biosafety levels (BSL) and the role of isolated yeast-like fungi.

Species and authors name*	Role*	BSL**
Candida albicans (Robin) Berkhout	sap, path	BSL-2
Candida krusei (Castellani) Berkhout	sap, path	BSL-2
Candida tropicalis (Castellani) Berkhout	sap, path	BSL-2
Debaryomyces castellii Capriotti	sap	und
Debaryomyces hansenii Lodder & Kreger-van Rij	sap, path	BSL-1
Debaryomyces mellissophilus Kurtzman & Kreger-van Rij	sap, path	und
Debaryomyces polymorphus Price & Phaff	sap	und
Debaryomyces pseudopolymorphus Price & Phaff	sap	und
Kluyveromyces polysporus van der Walt	sap	und
Lipomyces starkeyi Lodder & Kreger-van Rij	sap	und
Metschnikowia pulcherrima Pitt & M.W. Miller	sap	und
Pichia guilliermondii Wickerham (anamorpha = *Candida guilliermondii* Langeron&Guerra)	sap, path	BSL-1
Rhodosporidium malvinellum Fell & Hunter	sap	und
Stephanoascus farinosus de Hoog, Ranio-Lehtimaki&Smith	sap	und
Trichosporon ashii Akagi ex Sugita, Nishikawa & Shinoda	path	BSL-2
Trichosporon asteroides Ota	path	BSL-2
Trichosporonoides oedocephalis Haskin & Spencer	path	und
Trichosporonoides spathulata de Hoog	path	und
Yarrowia lipolytica van der Walt & von Arx	sap	und
Oosporidium sp.	sap	und
Schizosaccharomyces sp.	sap	und

path – pathogenic, sap – saprophytic, und – BSL undefined;
*according to Kurtzmann & Fell [4];
**according de Hoog et al. [2].

medicine [8]. Currently the medical leech is believed to be a small "pharmacological laboratory" (it is used for the production of many enzymes, a major one being hirudin). In turn, in medicine leeches cultured under sterile conditions are used in hospitals to support treatment of numerous diseases (e.g. hypertension, heart diseases, difficult-to-heal wounds).

The medical leech is found in small water reservoirs: ponds, peat ponds, lakes, and reservoirs situated among meadows, in old riverbeds and ditches. Less frequently it can be found in slow flowing rivers and streams. Overgrown wet banks that afford possibilities for placing cocoons are supportive for its presence as well [7, 9, 10].

It is a species with specific trophic and habitat requirements. Owing to its pharmaceutical and medical value, medical leeches should be protected together with entire biocenoses because species protection itself is frequently insufficient. That is why it seems that in the case of medical leeches, zone protection and establishing natural sanctuaries in the places of its presence would offer the best solution.

As in recent times, the use of *H. medicinalis* in contemporary medicine has increased again, microbiologically clean individuals should originate only from legal, controlled breeding stations. In the available literature, there is no data on the isolation of fungi from body covers of leeches and from their gastrointestinal system, but in our own studies the presence of fungi, including those that are dangerous pathogens for humans and animals, was confirmed. The most common pathogen is *Aeromonas hydrophila* [11, 12] but infection with *Vibrio fluviatilis* has also been reported. The incidence of wound infections has been reported to reach 7% and 20%. Multiple potential pathogens have been isolated from *H. medicinalis*, including *A. hydrophila, Staphylococcus species, Alcaligenes species, Pseudomonas putida* and *Fusobacterium varium* [13].

Finding fungi on covers and jaws allows formulating a hypothesis treating leeches as vectors of transmission of fungi present in the aquatic environment. At the same time, the absence of fungi in further segments of the gastrointestinal tract seems to be caused by excretion of many biologically-active substances, in particular hirudin, prostaglandin, hyaluronidase, lipase and neurotransmitters. Hyaluronidase is claimed to be a substance with double activity important for the analyzed problem of the presence or absence of fungi. On one hand it is a substance possessing strong antibiotic properties but on the other it is an enzyme capable of dissolving compounds of polysaccharides forming membranes of spores of numerous microorganisms. The cell membrane of yeast-like fungi is constituted by polysaccharides – mannan and glucan [14], which enables speculating that hyaluronidase may be a reason behind the absence of yeast-like fungi in the gastrointestinal tract of leeches which, having decomposed polysaccharides, weakens the skeleton of the cellular membrane. This in turn evokes inhibition of natural intracellular transformations in fungi and, probably, cell atrophy.

Fungi are a group of indicatory microorganisms indicating the presence of organic matter of various origin in waters [15-19].

Isolation of anthropopathogenic fungi from waters suggests that they are their natural reservoirs likely to represent an important source of fungal infections [20]. Bodies of leeches can be such a source in the analysed case. They may serves as transmitters of infectious microorganisms including potentially-pathogenic fungi.

When it comes to pathogenic yeast-like fungi, 3 species of the genus *Candida*: *Candida albicans, Candida krusei* and *Candida tropicalis* were isolated in the study.

Candida krusei and *Candida tropicalis* were isolated, for example, from lesions found in all tissues and organs of the human body [21].

Under the auspices of the European Confederation of Medical Mycology the updated classification of biological safety of fungi potentially pathogenic for humans and animals (BSL - biosafety levels) was introduced in 1996. According to that classification, 3 BSL levels were identified [22]. *Candida albicans* frequently isolated from polluted waters was classified, among other pathogenic species, to BSL class 2. The genus *Candida* fungi proved very useful in detecting contamination of water with faeces. This is the species that offers a more credible assessment of contamination than the application of "coli titre" [15]. According to Dynowska [16], that species can be potentially applicable as an indicator of trophic status and contamination of waters important in epidemiology of mycoses, and can be useful in sanitary-hygienic assessment.

Conclusion

In the context of the results obtained and in the case of prospective use of hirudotherapy, the problem is whether sterility of breeding stations and health status of the leeches are sufficient and whether they offer certainty and safety to the patient. Isolation of such a large number of pathogenic fungi from leeches poses a potential hazard to people who could have contact with non-sterile therapeutic material, which in this case the leeches are. According to the principle of Hippocrates, or rather Imhotep, *primum non nocere*, one of the supreme ethical principles in medicine, the reasonability of means applied for obtaining expected results of treatment should always be considered [23].

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