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INFECTIVE AGENTS IN FIXED ANATONICAL MATERIALS

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Abstract. The program of pre-graduate and postgraduate medical education involves the use of cadavers to teach anatomy by autopsy or examination of dissected samples. The preservation process of the biomaterial must ensure that it is free from damage, destruction and decomposition. Preservative solutions are used for this, but it remains unclear whether there is any risk of spread. microorganisms during the teaching of anatomy, research and autopsy procedures of fixed corpses. The results showed that the fixed cadaveric material, as well as preservative solutions, contain viable micromycetes. The study of the pathogenicity factors of fungi isolated from anatomical preparations showed that more than 80% of fungal strains have hydrolytic activity. This study highlights the importance of developing safe manipulation protocols to avoid possible infection and illness of staff and students.

Keywords: anatomical preparations, fungi, pathogenicity factors, enzymes

ИНФЕКЦИОННЫЕ АГЕНТЫ В ФИКСИРОВАННЫХ АНАТОМИЧЕСКИХ МАТЕРИАЛАХ

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Резюме. Программа додипломного и последипломного медицинского образования предполагает использование трупов для обучения анатомии путем вскрытия или изучения прозектированных образцов. Процесс консервации биоматериала должен гарантировать отсутствие его повреждений, разрушения и разложения. Для этого используются консервирующие растворы, однако остается неясным, существует ли какой-либо риск распространения микроорганизмов во время преподавания анатомии, исследований и процедур вскрытия фиксированных трупов. Результаты показали, что фиксированный трупный материал, а также консервирующие

растворы содержат жизнеспособные микромицеты. Изучение факторов патогенности грибов, выделенных из анатомических препаратов, показало, что более 80% штаммов грибов обладают гидролитической активностью. Это исследование подчеркивает важность разработки протоколов безопасных манипуляций во избежание возможного заражения и заболеваний персонала и студентов.

Ключевые слова: анатомические препараты, грибы, факторы патогенности, ферменты

INTRODUCTION

The human body has been used to teach anatomy since the Renaissance. In fact, the ancient Greek roots of the word “anatomy” mean “to cut” or “to separate”. Anatomy museums hold specimens of scientific interest. This excellent educational tool is used by anatomists when teaching future physicians. Modern methods of embalming human cadavers involve the use of formalin with the addition of glycerin, salts, disinfectants, and water. The embalming procedure should provide good preservation of organs and tissues with minimal structural changes to the corpse. Formalin is known to be a strong disinfectant. Its ability to inhibit the growth of bacteria and fungi even in low concentrations has been noted. This is due to the ability of formalin to attach to amino groups of protein molecules, which leads to changes and destruction of the molecular structure of proteins and nucleoproteins. It provides tissue hardness and inactivates enzymes responsible for postmortem autolytic processes [1–5].

Despite high efficacy and a wide range of antimicrobial activity, some microorganisms do not lose viability on formalin-treated surfaces, and some strains can even multiply in cadaver tissues [1]. For example, *Yaragalla* and *Rajput* found *Penicillium, Trichophyton* and *Aspergillus* species on skin and bone tissue of cadavers fixed with 5% formalin solution [6]. However, few studies have addressed the biological risk of manipulation of fixed cadaver and the spread of pathogens. N.A. Osman et al., S. Hayashi et al., Tabaac et al. showed the presence of pathogenic bacteria in 10 fixed cadavers used in practice for teaching anatomy: *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes* [7, 8]. The presence of fungal species such as *Trichophyton, Microsporum, Candida* and *Cryptococcus* was also noted in the work of C. Molina et al. [9].

Another important aspect is the discoloration of anatomical preparations under the influence of fungal metabolites, which leads to their unusability. The sources of contamination of cadavers with various species of microscopic fungi are quite diverse and include room air, personnel and students on the department.

AIM

The aim of the present study was to investigate the fungal species in the air of the anatomy laboratory and to determine the presence of formalin-resistant fungi on the surface of cadavers and to evaluate their biological activity.

MATERIALS AND METHODS

Material was collected with sterile cotton swabs from anatomical preparations, from solutions for storing wet preparations and from cadaver preservation baths. Air sampling was carried out by artificial sedimentation. Samples were sown on Czapek’s medium with subsequent cultivation at 28 °C (5–7 days). Species identification of fungi was carried out on the basis of cultural and morphological properties.

Taxonomic characters (morphology of reproductive structures, culture characteristics of colonies) were used to identify fungi. The names of fungal species are given according to the nomenclature of the Mycobank database [10].

Fungi isolated from anatomical preparations, wet preparation storage solutions, and cadaver preservation baths were analyzed for their ability to produce extracellular enzymes on solid medium. Protease activity was assessed by the formation of transparent zones around fungal colonies on skim milk medium [11]. To determine lipase activity, fungi were cultured on medium with Tween-80. Their enzymatic activity was evaluated by the formation of calcium oleate crystals. Hemolytic activity was evaluated by the formation of hemolysis zones on blood agar.

RESULTS AND DISCUSSION

The following micro-mycetes were isolated from the different materials.

- Air: *Aspergillus niger* Tiegh., *Penicillum lilacinum* Thom, *Aspergillus restrictum* Smitt, *Mucor ramosissimus*, *Aspergillus ustus* (Bainier), *Aspergillus flavus* Raper and Fennell, Gams, *Scopulariopsis brevicaulis* (Sacc.) Bainie, *Aureobasidium* spp., *Glioclanium* spp., *Trichoderma polysporum* Rifai.
- Solutions and flushes from cadaver preservation baths: *Gliocladium roseum* Bainier, *Gliocladium* spp.

Table 1

Enzymatic and hemolytic activity of the studied fungal species

Таблица 1

Ферментативная и гемолитическая активность изученных видов грибов

Виды грибов / Types of mushrooms	Количество штаммов / Number of strains	Количество штаммов, проявляющих гидролитическую активность / Number of strains exhibiting hydrolytic activity		
		протеаза / protease	липаза / lipase	гемолиз / hemolysis
Aspergillus ustus	5	2	1	2
A. nidulans	1	1	0	1
A. niger	3	3	3	3
A. restrictum	2	0	0	1
A. flavus	3	3	1	3
Gliocladium roseum	2	2	0	2
Trichoderma polysporum	4	3	4	2
Scopulariopsis brevicaulis	1	1	1	0

- Anatomical preparations: *Aspergillus nidulans (*Eidam), *Fonsecaea pedrosoi* (Brumpt).

It is interesting that the micromycete species isolated from the air environment of the anatomical laboratory were not detected in solutions or on anatomical preparations. Formalin-fixed preparations were covered with dense substrate mycelium. As a result of exposure to formalin vapors and low air exchange, no fungal growth occurred and no aerial mycelium was detected on the preparations. Thus, no contamination of the air environment with spores of fungi of the genus *Gliocladium,* as well as species of *A. nidulans* and *F. redrosoi* were recorded. Numerous metabolites produced by these fungi are dangerous for anatomical preparations. These are primarily quinones, which form stable complexes with natural polymers. As a result, anatomical preparations acquire coloration. In addition, volatile toxic isocyanates are formed, which are the main cause of the development of asthmatic status.

25 mycelial fungi isolated from anatomical preparations, wet preparation storage solutions and cadaver preservation baths were analyzed for their ability to produce extracellular enzymes on solid media. The following fungal species were tested: *Aspergillus niger lilacinum*, *A. nidulans*, *A. flavus*, *A. restrictum*, *A. ustus, Penicillum*, *Mucor ramosissimus*, *Scopulariopsis brevicaulis*, *Aureobasidium* spp., *Trichoderma polysporum, Gliocladium roseum*, *Fonsecaea pedrosoi.*

The study of the pathogenicity factors of micro-mycetes isolated from anatomical preparations showed that more than 80% of fungal strains have hydrolytic activity (Table 1).

Representatives of *Penicillum lilacinum, Mucor ramosissimus* and *Fonsecaea redrosoi* species showed no hydrolytic activity.

An important factor in the pathogenicity of such fungal species as *Aspergillus ustus, A. niger, A. flavus* are proteases. The study of virulence factors of these fungi has shown that the main role in the development of pulmonary aspergillosis belongs to proteolytic enzymes — elastases, collagenases [12]. The ability to form extracellular phospholipases has been revealed in many opportunistic fungi that are causative agents of mycoses [13].

A pronounced hemolytic activity was found in 56% of fungal isolates. It has been proved that in a number of mycelial fungi hemolytic activity is the result of toxin production. Thus, S.J. Vesper et al. isolated the hemolytic agent stachylysin from *Stachybotrys chartarum* [14].

CONCLUSION

Thus, hydrolytic enzymes, which are pathogenicity factors, were detected in the majority of tested fungal cultures. Effective prevention of contamination of anatomical museums and departments by mycelial fungi is necessary, as it poses a serious health hazard to personnel.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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