Central Asian Journal of Medicine

DERMATOMYCOSES AND METHODS OF LABORATORY DIAGNOSIS

Egamberdi E. Eshboev[1], Otabek S. Imamov[2], Normurod D. Djumaev[3], Indira N. Abduvakhitova[3], Gayrat Sh. Toxtayev[4]

1 Professor, Republican Specialized Scientific-Practical Medical Center of Dermatology and Cosmetology, Tashkent, Uzbekistan

2 Associate Professor, Department of Dermatovenereology and Cosmetology, Tashkent Medical Academy, Uzbekistan

3 Department Head, Republican Specialized Scientific-Practical Medical Center of Dermatology and Cosmetology, Tashkent region branch, Uzbekistan

4 Assistant, Department of Dermatovenereology and Cosmetology, Tashkent Medical Academy, Uzbekistan
E-mail: gayrat.uz@mail.ru

ABSTRACT

The purpose of the study: preparation of a cheap and high-quality fresh medium, the basis of which is made up of local ingredients for the growth of pathogenic fungi. Materials and research methods: in the process of carrying out scientific research work, pathological material from 400 patients (skin scraping, damaged hair fiber, and nail plates) was studied in the Saburo food environment, which is applicable to the causative agents of fungal diseases of the skin, and in the proposed food environment. The results: cheap and high-effective medium was created for use in the daily practice of bacteriological laboratories under all treatment (dispensaries of venereal diseases, private laboratories) institutions located in the territory of the Republic.

Key words: dermatomycosis, fungi, Saburo, laboratory, medium.

INTRODUCTION

By the 21st century, fungal infections have caused a variety of diseases among humans, including, within the animal world. According to data provided by the World Health Organization, one in five of the inhabitants of the globe suffers from one type of fungal disease (4,21). Especially their development against the background of secondary processes in other diseases, primary diseases with

various Genesis, has severe consequences. An example of this is AIDS, and COVID-19 diseases (1,17). Fungi are the main cause of death of patients with severe pathologies – diseases accompanied by cancer, autoimmune, and immunodeficiency (8). It is worth saying that in conditions of immunodeficiency of conditionally pathogenic fungi, their pathogenic properties are increasing. New nosological forms are emerging, in addition, the resistance of fungi to antimycotic drugs is increasing. It should not be forgotten that the products of the life-activity of fungi, for example, mycogenic allergies, and mycotoxicoses, have a huge impact on human health.

Dermatomycoses in the English-language scientific literature ringworm or tinea (English "ring" – round or ring–shaped; "worm" – worm, snake); ringworm – contagious lesions of the skin with pathogenic fungi in a ring-shaped form; Latin "tinea" is a fungal disease of the skin, especially the head-haired part. This is the case when humans and animals are damaged by fungal infections of keratin-retaining tissues (skin, fine hairs, hair, and nails) (5). The pathogens of dermatomycosis damage the keratin-retaining tissues of humans and animals at the expense of the activity of its keratinase enzyme. Dermatomycoses are considered fungi-Cosmopolitan and are distributed in all regions of the globe. They are pathogenic to humans, infecting all domestic and wild animals and fish, and birds (14). Pathogenic species of fungi constitute a large group of disease-causing microorganisms in humans and animals. In general, more than a hundred species of all fungi are pathogenic, and the bulk of the rest is conditionally pathogenic. They belong to the genus of simple plants in terms of structure and are distinguished from plants by the absence of the substance chlorophyll in their composition. Fungi are common in nature and are found mainly in moist soil, watershed areas, in the air, in the bodies of plants, and trees, in the animal world, and finally among humans (20).

Most types of pathogenic fungi are found in an anaerobic environment, that is, they prefer conditions with little oxygen. For their survival, nitrogen, carbohydrates, and minerals are necessary, favorable environmental conditions for them are pH=6.0-6.4, and a moderate temperature is 220 -370 C. Fungi have round, oblong, tubular, horny forms, the length of their bodies, that is, their mycelium, is from 4-5 μm to 60-70 μm. (3). For fungi to live, reproduce, and function, they are required to have conditions, such as relatively low temperatures, darkness, humidity, an oxygen-free environment, and high levels of carbohydrates. On the contrary, dry temperatures, light, oxygen-rich conditions, and high heat levels are considered unfavorable. Fungi reproduce mainly by division or budding.

They have the characteristics of forming colonies that are specific in artificial planting areas, that is, in nutrient environments (12).

It is worth saying that medical mycology has long been overshadowed by the science of bacteriology and virology and is considered a secondary level to it. But changes in immunology, dermatology, endocrinology and infectious diseases require a new look at medical mycology. Particular concerns are the reproduction of conditionally pathogenic systemic fungi that occur against the background of underlying diseases and further complicate their course. Global problems are increasing the number of invasive fungi, and the collaborative arrival of fungi among people infected with HIV or COVID-19 is increasing their role. (7,9,18) It is noteworthy that in Endocrinology, organ transplantation, onco-hematology, and neonatology, the range of negative effects of pathogenic fungi increases. It is worth saying again that skin diseases caused by pathogenic fungi – with dermatomycoses (greek "derma" – skin, "mycosis" - fungi) (leg paw mycosis, rubromycosis, onychomycosis, trichophyty, microsporia, etc.) The number of patients is increasing in arithmetic progression (2).

The process of formation of dermatomycoses depends on the degree of disease initiation of fungi and the immunobiological characteristics of the microorganism, the age, and gender of patients, and the state of their endocrine glands. An important role in this is played by the increased absorption properties of the epidermis and dermal layer, profuse sweating, and the specific chemical composition of the skin (15).

The condition of occurrence of fungal diseases among children, especially in school-age children is because of insufficient levels of keratin in the structure of the skin epidermis and hair cells, the transition of the skin's water-oil-chemical environment to an alkaline or moderate state leads to the activation of fungi and an increase in the level of virulence. Dermatomycoses are more common, especially in patients with chronic and infectious diseases and debilitated. The source of the disease is a sick person or infected animals, from which the disease to healthy people occurs directly as a result of household contacts or indirectly, that is, items that are in the consumption by patients (clothes, dishes, toys, etc.). Is transmitted through. After the disease is transmitted, its clinical signs are manifested and develop on the skin, hair, nails, and mucous membranes (6).

Medical mycology faces serious problems in the following years, a number of antimycotic drugs have been developed and put into practice in order to properly treat the disease. But the need to improve laboratory examination methods is increasing from year to year. Because insufficient laboratory data often leads to a late receipt or incorrect execution of the examination results, ultimately this

condition complicates the treatment work and leads to a chronic course of the disease (10,11).

Timely early diagnosis significantly increases the likelihood of successful recovery, therefore, from the very beginning, it is necessary to carry out Mycological examinations of the patient, assuming the presence of a fungal infection. The identification of the causative agents of dermatomycosis of fungi from the affected skin or its excess provides the necessary opportunity for an accurate diagnosis. The development of molecular-genetic, biochemical, and immunological technologies began to be widely used in the laboratory diagnosis of fungal infections. But in order to fully study the morphobiological antimycotic properties of the etiological factor, it is necessary to isolate the culture of pathogens in food environments, in addition to the indication of mycotoxins, mycotoxicosis, and toxicoinfections of fungi. And for this, of course, it is necessary to introduce fungicides (22,25).

Various nutrient media have been proposed by a number of authors to obtain a pure culture of dermatomycosis pathogens (17,23). Their main components are peptone, agar-agar, and products such as various amino acids. But, due to the extremely high cost of the ingredients that make up the composition of these food media, as well as its deficiency and the growth of disease pathogens in these food environments in the long term (21-28 days), treatment was practically not used in the mycological laboratories of preventive institutions. So the problem remains open.

Taking into account the above points of view and feedback, as well as the relevance of the work, we aimed to prepare an inexpensive and high-quality new food environment, the basis of which is composed of local ingredients, in order to extract the causative agents of dermatomycosis and extract its pure culture.

It is known that the microscopic diagnosis of dermatomycoses does not allow for to the determination of the type of disease pathogens (21). The correct diagnosis of the patient can be made by determining the type of pathogens of diseases by absorbing the causative agents of fungal diseases of the skin in a cultural style, that is, in special nutrient media. At the same time, it is possible to determine the state of susceptibility to antimycotic drugs to the identified causative agent of the disease.

In the cultural diagnosis of dermatophytes, frequent interruptions in supply due to the fact that the food media used in today's practice are imported from foreign countries, and the high cost of the food environment causes a number of disadvantages. Alternatively, in these food environments, the pure culture of dermatophyte pathogens is isolated in the long term (21-28 days), while the level

of reproduction of fungal fungi in most cases does not exceed 40% in the practice of reputable Mycological laboratories (13.24).

It is known that in the Saburo food environment, the level of isolation of the pure culture of fungi remains low. In the Russian Federation, the level of isolation of the pure culture of fungi in the cultural investigations carried out in Saburo food environments in subsequent years did not exceed 36% (16).

This figure was 30-32% in the research carried out in the Republic on the examination of fungi by planting them in the Saburo food environment.

After planting in the nutrient medium, the growth of bacteria begins from 2-3 days, the growth of yeast and mold fungi from 4-5 days, and the growth of fungi begins from 6-7 days. Secondly, the causative agents of dermatomycosis (trichophytia, microsporia, epidermophytia) have high nutritional needs. In order for most types of dermatophytes to grow in their nutrient medium, a complete set of vitamins and proteins of animal origin is initially required. Therefore, the remoteness of the identification days of dermatophyte pathogens planted in the current Saburo food environment (19) once again requires conducting scientific and practical research in this regard.

The objective of this research is to produce a new nutrient medium to extract pure cultures of fungi from clinical materials that satisfy the efficacy criteria necessary to ensure the accurate and objective results required for the reliable results of any clinical mycological examination.

The composition of the proposed new nutrient medium consists of the following components (g/l): distilled water – 1 L, mycological peptone – 10.0, bacteriological agar-agar – 18.0-20.0, keratin hydrolysate – from 10.0 to 20.0, apricot extract – 80.0, vitamin V1 (thiamine chloride) – 1.0, ciprofloxacin – 5.0, cycloheximide – 0.001.

Microbiological peptone-proteins, carbohydrates, vitamins, as well as various minerals, are necessary for the nutrition of fungi. Most dermatophytes grow well in peptone food environments with added carbohydrates. The proper combination of carbohydrates and peptone added to the food environment is one of the important factors in the formation of the culture of dermatophytes. The presence of a sufficient amount of peptone reduces cases of pleomorphic changes, layering, twisting, and crater-shaped formation in the colony of dermatophytes.

The study of the assimilation of dermatophytes of proteins and peptones was explained by the fact that these substances can be sources of carbon and nitrogen.

Bacteriological agar – agar is a plant substitute for gelatin in the form of a powder or plate obtained from a mixture of agaropectin and agarose polysaccharides. Agar-agar does not contain fats at all, consisting of 5% protein

and 95% carbohydrates, while being a product rich in minerals necessary for the absorption of dermatophytes such as magnesium, iron, calcium, and iodine.

Keratin hydrolysate is a natural protein obtained from the horns of sheep and goat wool and poultry feathers of small and large horned animals. It consists of peptides, polypeptides, and amino acids.

Keratin is a natural protein, the skin and its derivatives are the main components of fine hairs, hair, and nails. Does not melt in a natural state. Through hydrolysis, large molecules of keratin are transferred to the state of a water-soluble substance. Keratin hydrolysate is a product rich in amino acids that dermatophytes need to grow.

The use of keratin tissue for growing dermatophytes in artificial food environments occurs in the practice of mycological examinations. With a gradual dissolution of the keratin substance in the structure of keratin-holding tissues under the influence of dermatophytes, changes in the skin, including hair, the appearance of wounds on the surface of the skin are associated with the assimilation of keratin by dermatophytes, the main mass of the skin horn layer.

By adding keratin hydrolyzate in sufficient quantities to the food environment, the need for dermatophytes for amino acids is compensated, which in turn ensures that fungi grow faster in the food environment of their aggressors.

Dermatophytes synthesize proteins necessary for cell structure from simple amino acids. The growth of dermatophytes in the nutrient medium is due to the fact that it digests a mixture containing a mixture of several natural amino acids, in such conditions amino acids are absorbed directly, so the need for them for the formation of proteins in cells is less. Many amino acids are lightly assimilated by most dermatophytes.

Apricot extract is an inexpensive, natural, local product added to the food environment and is very rich in carbohydrates, amino acids, and minerals necessary for the growth of dermatophytes. Apricot extract contains amino acids such as arginine, glycine, lysine, tryptophan, isoleucine, and tyrosine. 100 grams of apricot extract contains 65 gr. there are minerals carbohydrates, 1162 mg potassium, 55 mg calcium, 27 mg iron, and 10 mg sodium.

The addition of apricot extract, a complex of carbohydrates that are natural in their origin in the food environment, is important for the nutrition of many dermatophytes. In addition to the fact that apricot extract is extremely rich in carbohydrates, the saturation of dermatophytes with the minerals necessary for its nutrition further enriches the effectiveness of the nutrient medium.

The addition of the antibiotic **ciprofloxacin** to the nutrient medium was used to suppress the growth of the bacterial flora and thereby eliminate the antagonistic effect on dermatophytes.

Cycloheximide- inhibits the growth of yeast and mold fungi in the food environment.

New food environment preparation techniques: Based on the information given above, all the required components are prepared in advance. Initially, 18.0-20.0 gr of bacteriological agar-agar is poured into a glass flask, 900.0 ml of distilled water is poured over it, and the mixture is heated until agar-agar dissolves. 10.0 gr of Mycological peptone ,10.0 gr to 20.0 gr of keratin hydrolysate are added to the dissolved mixture, the amount of the mixture is brought to 1 liter by adding distilled water, and the product is thoroughly mixed. The resulting mixture is shaken. After that, the nutrient medium is poured into glass containers. The mouth of the jars is closed with a cotton gauze stopper and sterilized in an autoclave at 1 atm (1200 C) for 20 minutes.

After sterilization, the nutrient medium is cooled, and before solidification, 80.0 g of apricot extract, 1.0 g of vitamin V1(thiamine hydrochloride), 5.0 g of ciprofloxacin, 0.001 gr – cycloheximide are added to it. Its environment is then equated to 6.5.

Preparation of apricot extract. To do this, 1 kg of dried sorrel of apricot is taken and first thoroughly washed in warm water. Then it is crushed in a sharp knife to the same size (0.3 – 0.5 cm), placed in 2.5-3.0 liters of distilled water, and boiled in an enameled container on a gas stove for 30-35 minutes over the same fire. When the required time is up, the decoction is cooled and passed through a cotton-gauze filter. The resulting clean solution is placed in glass jars, and their mouth is closed with cotton gauze plugs. Then sterilized in an autoclave at 1 ATM (1200 C) for 15 minutes. Sterile apricot extract from an autoclave can be safely stored in a cool laboratory place for up to a year.

The nutrient medium prepared according to the instructions described above is poured into sterile Petri Bowls in a volume of not less than 3 mm (10.0 ml) thick. The nutrient medium can also be poured into branded test tubes (3.0 ml) and used freely. It is better to keep Petri bowls and test tubes in sterile boxing conditions, in which the nutrient medium is poured.

Recommended "nutrient medium for growing dermatophytes" (invention patent No. JAP 07071.22-08-2022 y) the effectiveness of the current Saburo was carried out by model experiments with the nutrient medium and by the method of comparative analysis of the growth of fungodermatomycetes in clinical materials.

In the experiments carried out, the growth rate and character of the colonies, as well as the morphological characteristics of dermatomycetes, were studied.

For the study, materials from damaged smooth skin, hair, and nail plates of 400 patients (184 children under 14 years old, 107 men and 109 women) were planted parallel to the surface of the standard Saburo dense nutrient medium, and the proposed nutrient media at one time and incubated at a thermostat at 260-370s. In addition to it, the damage of the two nutrient media by bacterial, yeast, and mold fungi has been studied in a comparative way.

From the practice of mycological examinations, it is known that in cultural diagnosis, the negative effect of secondary bacterial infections, yeast, and mold fungi infestation of food media is great. Our experiments have shown that in the Saburo food environment, the infestation with bacterial, yeast, and mold fungi was 19-21% in the case, and in the proposed food environment 11-13%.

In our model research work, cultures of three species of fungi (Trichophyton rubrum, Microsporum canis, Epidermophyton floccosum) were studied, the most common among dermophytes. These types of dermatomycoses are fungi that can be isolated from most patients. Strains of skin-venereal diseases of the Tashkent region from the collection of therapeutic and pathogenic cultures of the Mycology Laboratory and pure cultures extracted from pathological materials obtained from patients diagnosed with mycosis were also used.

The growth dynamics of dermatophyte colonies were as follows. On the first day of observation, an increase in both environments did not occur, on the second day, the first 0.1-0.3 cm increase in Trichophyton rubrum began to be observed in the proposed food environment. On days 4-5-6, in Saburo and the proposed environments, the fungal colonies reached from 0.2 cm to 3.0-3.8 cm. But it is worth saying that by the 6-7 days of observation, the size of the colonies in the proposed food environment (6.1-6.5 cm) became almost twice as large as in the Saburo food environment (3.5-3.8 cm.

In the same way, a comparison was studied of the features of the formation of a colony of fungi of four species (Trichophyton rubrum, Microsporum canis, Epidermophyton floccosum, Trichophyton verrucosum) in parallel at one time. From our research, it is known that in the proposed food environment, the flourishing and colony formation of all fungi was two times less (from 7-8 days to 11-12 days) than in the controlled ones.

Identification of colonies of fungi-dermatophytes in the food environment plays a key role in the accurate diagnosis of dermatomycoses. In this part of the study, studies were carried out on the identification of the colonies of fungi-dermatophytes belonging to four species (Trichophyton rubrum, Microsporum

canis, Epidermophyton floccosum, Trichophyton verrucosum). From the research, it turned out that Trichophyton verrucosum from research cultures was formed in 11-12 days, and Epidermophyton floccosum and Trichophyton rubrum in 9-11 days at the level of identification (pigment formation, mycelium, and the appearance of spores, etc.) in the short term and made it possible to fulfill the task of effective collection.

On the contrary, it was noted that fungi of four species, planted in the Saburo food environment, grow for 18-26 days. Hence, in relation to the proposed nutrient medium, the growth time of dermatomycetes was two times longer.

Recommendations and conclusions:

- In the practice of Mycological laboratories of the Republic, it was shown that the rate of absorption of the pure culture of dermatophytes of the food environment used is 30-32%, and the growth period is 18-21 days;

- For the first time, a quality new nutrient medium was prepared and recommended for the collection of dermatophytes, the basis of which is made up of local ingredients;

- It has been proven that the newly prepared food environment is effective from the environment used in practice, including the growth rate has been reduced from 6-7 days to 2-5 Days, and the identification period has been reduced from 18-21 days to 8-12 days;

- Secondary bacterial, yeast, and mold fungal infestation in the proposed food environment was reduced from 19.0% -21.0% to 11.0% -13.0% compared to the standard Saburo food environment;

-All technical requirements and guidelines of the proposed food environment were developed and recommended by standards.

REFERENCES

1. Adaskevich V.P. Skin manifestations in patients with coronavirus infection covid-19 and features of the work of a dermatovenereologist during a pandemic CONSILIUM MEDICUM 2020 | volume 22 | №7 / page 13.

2. Abidova Z.M., Nurmatov U.V., Tulyaganov A.R. Species composition of dermatophyte pathogens for 6 years (1995-2000) according to the data of the of the Ministry of Health of the Russian Federation. In the book Theses of the VIII All-Russian Congress of Dermatovenerologists M. -2001, -part I, -p. 154-5.

3. Allaberganova Z. S. Cultivation of yeast-like fungi of the genus CANDIDA on different nutrient media and their biological properties, abstract dissertation Candidate of Biological Sciences, Tashkent, 2006

4. Antonova S. B. MODERN CLINICAL AND EPIDEMIOLOGICAL FEATURES OF THE INCIDENCE OF DERMATOMYCOSIS IN CHILDREN. OPTIMIZATION OF DIAGNOSTIC, MEDICAL, AND PREVENTIVE TECHNOLOGIES Dissertation for the degree of Candidate of Medical Sciences, Yekaterinburg, 2019, pp. 4-5.

5. Arifav S.S., Eshboev E.X. skin and venereal diseases, Tashkent, 1997.

6. Arifov S.S. Clinical dermatology and venereology, Atlas, Tashkent, 2008.

7. Dvoryankova E.V., Korsunskaya I.M., Slavyanskaya T.A. Skin manifestations of COVID 19 Bulletin of the RUDN. Series: MEDICINE 2021;25(1): pp. 13-14

8. Report on the global HIV epidemic/AIDS. Global Report. 10th. UNAIDS 2020.

9. Zykov K.A., Stadnikova A.S., Tamrazova O.B., Sinitsyn E.A. Skin manifestations in COVID-19. Clinical dermatology and venereology. 2021;20(4):50 54.

10. Imamov O.S., Correction of connective tissue metabolism in patients with mycoses of the feet of elderly and senile age: abstract. dis. Candidate of Medical Sciences, Tashkent, 2011.

11. Karabaeva I.T. Development of methods of treatment of zooanthroponosis microsporia on the basis of clinical and immunobiochemical research, abstract of the dissertation of Doctor of Philosophy (Ph.D.), Tashkent, 2019.

12. Kochneva E.V. Determination of pathogenicity factors of Candida albicans fungi and their role in the development of the infectious process. Topical issues of modern medicine: Collection of scientific tr. Yekaterinburg, 2014: 110-113.

13. Podkhomutnikova O.V., Vorobyeva O.N., Konyakhina I.G., Lazareva G.A., Tipikina L.M. Method of isolation of dermatophytes from clinical material. RF Patent 2181144 (10.04.2002). 7 From 12 N 1/14, From 12 Q 1/04.

14. Raznatovsky K.I., Rodionov A.N., Kotrekhova L.P. Dermatomycoses: A guide for doctors. - St. Petersburg: Publishing House, 2003, p. 158.

15. Sadikov A.A., Tokhtaev G.Sh., COMPARATIVE ANALYSIS OF THE STATE OF THE SKIN MICROBIOME IN ATHLETES OF VARIOUS SPECIALIZATIONS DURING TRAINING PERIODS, Dermatovenerology and reproductive health 2020 No. 3-4, ISSN 2091-5969., 109 p.

16. Sergeev A.Yu., Sergeev Yu.V. Fungal infections. A guide for doctors. – Moscow:, 2008. – 480 p.

17. Tashkenbayeva U.A., Klebleeva G.D. Features of trace element and immunological status in patients with dermatological manifestations after COVID-

19 infection COLLECTION OF ABSTRACTS OF THE SCIENTIFIC AND PRACTICAL CONFERENCE "DERMATOVENEROLOGY AND COSMETOLOGY: ACHIEVEMENTS OF SCIENCE IN PRACTICE" 2022 Page 51.

18. Tilovberdiev Sh.A. Deep Mycoses in immunocompromentive patients: clinic, diagnosis, treatment, and Prevention; abstract of the dissertation of Doctor of Medical Sciences, Tashkent, 2020.

19. Khismatullina Z.R., Mukhamadeeva O.R. Sposob videlenia dermatofitov. Vestn dermatol 2006; 2: 25-27.

20. Eshbaev E.X., Babajanov X.R., 2019 M. Khudoynazarov S.Q infectious and parasitic diseases of the skin and their laboratory diagnostics, Tashkent, 2021.

21. Eshbaev E.X., Mirsaidova M.A. Laboratory diagnosis of the diagnosis of dermatovenerological diseases. Educational guide, Tashkent 2022.

22. Yakshibaeva L.A., Knyazeva O.A. DERMATOMYCOSES: FEATURES OF DIAGNOSTICS AND THERAPY // European Journal of Natural History. – 2021. – No. 2. – p.51.

23. Garg J., Tilak R., Garg A., et al. Rapid detection of dermatophytes from skin and hair. BMC Research Notes 2009; 2:60- 66.

24. Kwon-Chung K.J., Bennett J.E. Medical Mycology. Lea & Febiger. Philadelphia – London, 1992.- 866 p.

25. Rippon J.W. Medical mycology. In The Pathogenic Fungi and the Pathogenic Actinomycetes 3rd edition. Philadelphia: WB Saunders; 1988. 14.