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THE MICROBIOLOGICAL STRUCTURE OF OTOMYCOSIS: SENSITIVITY PROFILE OF AGENTS TO ANTIFUNGAL DRUGS

Vladyslav A. Smiianov, Tetiana V. Ivakhniuk, Inna O. Plakhtiienko, Yevhen V. Smiianov, Polina O. Hornostaieva

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ABSTRACT

Aim: To study the species composition of microorganisms isolated from patients with otomycosis, and to control the sensitivity of isolated microorganisms to the most commonly used antifungal drugs.

Materials and methods: The main group of study was 132 patients with a preliminary diagnosis of Otomycosis was carried out in the period 2020-2022.To study the sensitivity of isolated microorganisms to antifungal drugs, the Himedia paper disk method (India) was used.

Results: Analysis of studies showed that among 132 patients, fungal infection was found in 101 patients (76%), the cultural method – in 31 patients (23.5%); of them women – 56 (42.4%), men – 60 (45.5%) aged 16 to 76; children – 16 (12.1%) aged 6 to 12 years. However, among all patients (n = 132) with otomycosis, fungal lesions of the outer ear prevail, which were detected in 85 (64.4%) patients, that is, fungal otitis was detected in 47 (35.6%).

Conclusions: The general structure of the species spectrum of etiologically significant pathogens of otomycosis: Candida spp. (78.0%) of the total spectrum of isolates. The sensitivity of isolated micromycetes to antifungal drugs varied in different fungal species and in different antifungal drugs.

KEY WORDS: otomycosis, microflora, microbiota, mycobiota, antifungals, resistance

INTRODUCTION

The issues of microbiological control of the species composition of the microflora of the ear in patients with otomycosis are reviewed in this article. Data on the sensitivity of isolated isolates of microorganisms to antifungal drugs are presented.

According to WHO, 20% of the world's population suffer from mycotic diseases of various locations [1]. The increase in the incidence of mycosis since the middle of the XX century is associated with many factors, including the introduction into medical practice of antibacterial drugs and steroids, which undoubtedly increased the number of not only candidiasis, but also mycoses caused by mycelium fungi [2, 3,]. An increase in the incidence of mycoses, infection and superinfection with fungi often contribute to the transition of acute processes to chronic, recurrence and more severe disease. Delayed diagnosis and irrational treatment can lead to generalization of fungal infection, which has a more severe course [4, 5].

AIM

In view of all of the above, the purpose of our study was to investigate the species composition of microorganisms isolated from patients with otomycosis and to monitor the sensitivity of isolated microorganisms to the most commonly used antifungal agents.

MATERIALS AND METHODS

A complex examination and treatment of patients (n = 132) with a preliminary diagnosis of "Otomycosis" in the period 2020-2022 were conducted. The patients were subjected to a comprehensive examination according to the standards of care orders of the Ministry of Health of Ukraine. The criterion for the diagnosis of mycotic ear lesions was a combination of careful collection of features of the clinical course of the disease, history, otoscopic changes and methods of microbiological laboratory diagnostics. The procedure for examining these individuals was in accordance with the standards of the ethics committee.

The criteria for the diagnosis of "Otomycosis" was a combination of the following features:

1. Presence of characteristic clinical picture.
2. The presence of fungus elements during microscopy of secretions.
3. Growth of the fungus on elective nutrient media (on Saburo medium and chromogenic agar, Himedia (India)) with determination of the degree of biological contamination.

To establish the etiological structure of the causative agents of ear inflammatory processes, a bacteriological and mycological study of the ears from the auditory canal was performed at the microbiological laboratory of Sumy State University (Ukraine) using classical methods of isolation and identification. To study the sensitivity of isolated microorganisms to antifungal preparations, the Himedia paper disc method Himedia (India) was used. The results of the studies were statistically processed. Graph Pad Quik Calcs was used to calculate the t-Student test.

RESULTS

Analysis of the studies showed that among 132 patients with inflammatory diseases of the ear (external otitis, acute or chronic perforative otitis media) fungal lesions were established taking into account the history, complaints of the patient and objective examination (otoscopically Fig. 1) in 101 patients (76%), culture method – in 31 patients (23,5%); of these women – 56 (42.4%), men – 60 (45.5%) aged 16 to 76 years; children – 16 (12.1%) aged 6 to 12 years. However, among all patients (n = 132), otomycosis is dominated by fungal lesions of the external ear, which were detected in 85 (64.4%) patients, meaning fungal otitis was detected in 47 (35.6%).

In total, 132 strains of the auditory canal fungi were isolated and identified in patients with otomycosis in a diagnostically significant amount (≥ 105 CFU / ml swab wash). The incidence of etiology of otomycosis in monoculture was 72.7% (96 isolates), and microbial associations were isolated in 36 patients (27.3% of the examined). The specific structure of otomycosis agents is presented in table I.

Considering the results of mycological study of the ears from the auditory canal of patients with otomycosis, it should be noted that during the invasion of blastospores of yeast fungi of the genus *Candida*, which according to the results obtained (Table 1) occupy the first place in the structure of causative agents of otomycosis (78,0%). It is established that these fungi can have both extracellular and intracellular location and the ability to reproduce in the epithelial cells of the macroorganism. Penetrating into the epithelial cell, *Candida* spp. parasites in its cytoplasm, using the substance of the host cell for its development. The pseudomycelia of the fungus can sprout right up to the cell nucleus, causing irritation of the cytoplasm and destruction of the nucleus. On the other hand, with prolonged persistence within the epithelial cell and even in its reproduction, *Candida* spp. are surrounded by a dense mucopolysaccharide microcapsule, which to some extent protects them from the effects of drugs [3, 5].

In addition to mycologic all patients with otomycosis, a bacteriological study of the material from the external auditory canal was performed: fungal-bacterial associations were detected in 27.3% of the observations. Analyzing the results of bacteriological and mycological methods of diagnosis, we found that the only pathogens of fungal etiology that were identified in association with bacterial microbiota were *Candida* fungi (Table 1), with these fungi isolated in the amount of ≥ 106 CFU / ml wash. Bacteriological examination of the composition of the microbiota washes from the external auditory canal of patients with otomycosis showed that the fungal-bacterial microflora was represented by two-component associations (58.3% of observations) and three- and four-component (33.3% and 8.4%, respectively) (Table 2), but the dominant species in the association were *Candida* spp. (*Candida* spp. total contamination rate was significantly ($p < 0.05$) higher than associate bacterial contamination rate).

It is found by us that in all patients with otomycosis caused by fungi of the genus *Candida* (65.0% of observa-

Table 1. Specific structure of the agents of otomycosis.

	The number of microorganisms isolated					
	in monoculture (n = 96)		in the microbial association (n = 36)		Totally (n = 132)	
Kind of microorganism	абс	%	абс	%	абс	%
Candida albicans	26	27,1	14	38,9	40	30,3
Candida tropicalis	10	10,4	7	19,5	17	12,9
Candida glabrata	8	8,3	1	2,8	9	6,8
Candida stellatoidea	8	8,3	3	8,3	11	8,3
Candida parapsilosis	8	8,3	11	30,5	19	14,4
Candida krusei	7	7,3	-	-	7	5,3
Aspergillus niger	17	17,7	-	-	17	12,9
Aspergillus flavus	3	3,1	-	-	3	2,3
Aspergillus fumigatus	2	2,1	-	-	2	1,5
Penicillium notatum	3	3,1	-	-	3	2,3
Penicillium chrysogenum	3	3,1	-	-	3	2,3
Mucor spp.	1	1,1	-	-	1	0,7

tions) lesions of the middle ear, which were characterized by the presence of white or liquid consistency of the white gingiva, perforations of the tympanic gut, were revealed in otoscopy. In 35.0% of cases, candidiasis did not have a classic clinical picture, which may be associated with the presence of associated fungal-bacterial otitis.

It is known that in microbial associations between different species complex and ambiguous relationships are formed, in which the mutual influences of the association members on each other and on the macroorganism are tightly intertwined. It is considered to be that such effects can take place in the following main directions: 1) the associative can change biological properties, stimulate or inhibit the reproduction and development of the main pathogen; 2) under the conditions of new interactions between microbes, their effect on the macroorganism can change, both by increasing the virulence of the pathogen, and due to the formation of new factors that aggravate the course of the disease; 3) additional human sensitization by microbes that make up the association.

In practice, physicians often conduct empirical therapy with antibiotics and / or antifungal drugs (antifungals), relying on the choice of drugs on world and local data resistance to these drugs. On the other hand, monitoring of antibiotic resistance shows a change in the resistance of pathogens to antibiotics, which are widely used in medical practice [1, 3, 5], the same tendency is observed among yeast and molds.

A necessity to study the species composition of bacterial (micro-, bacterial biota) and fungal (mycobiota) flora against suppurative inflammatory diseases of ENT organs and study the sensitivity of microorganisms to antibiotics and / or antifungals is an important condition for successful treatment of infectious pathology [6].

Sensitivity analysis of the investigated *Candida* fungi isolated from patients with otomycosis to a number of antifungal drugs revealed a high level of resistance to some of the antifungal drugs studied. The highest activity was demonstrated by voriconazole, to which 94.17% of isolated strains of *Candida* spp were sensitive. Relative to other antifungal drugs, a high percentage of resistant strains of *Candida* spp. ranged from 68.9% to 33.0%: 68.9% – to nystatin; 50.5% – to itraconazole; 48.5% – to ketoconazole; 34.95% – to clotrimazole and 33.0% – to fluconazole.

Due to the fact that in 27.3% of cases, according to the results of bacteriological and mycological studies of the material from patients with otomycosis, fungal-bacterial etiology of the disease was revealed, we performed an integrative assessment of the microenvironmental assessment of the microflora of the studied biolocus. The following indicators were used for the integral assessment of the microenvironmental characteristics of the microflora: Specific saturation index (IVN) – the average number of species included in the biocenosis; Sustainability index (C) – identification of the participation of different species in the structure of the biocenosis (C = (p / P) x100%, where C is the indicator of sustainability, p is the number of observations containing the investigated species; P is the total number of observations). The interpretation was carried out according to the following data: > 50% – permanent view; 25-50% – additional view; <25% is a random species of microorganisms.

Table 2. The composition of the microbiota (association) in otomycosis.

Microorganisms	Frequency of discharge from the ear	
	абс	%
Monoculture	96	72,7
Associations:	36	27,3
Two-component:	21	58,3
Candida spp. + S. aureus	11	52,5
Candida spp. + K. pneumoniae	4	19,1
Candida spp. + S. haemolyticus	3	14,2
Candida spp. + P. aeruginosa	3	14,2
Three-component:	12	33,3
Candida spp. + S. aureus + P. aeruginosa	5	41,7
Candida spp. + S. epidermidis + P. aeruginosa	3	25,0
Candida spp. + S. aureus + K. pneumoniae	3	25,0
Candida spp. + S. epidermidis + C. pseudodiphtheriticus	1	8,3
Four-component:	3	8,4
Candida spp. + S. epidermidis + E. coli + E. faecalis	2	66,7
Candida spp. + S. aureus + S. epidermidis + E. faecalis	1	33,3

Table 3. The resistance profiles of isolated *Candida* spp.

Kind of microorganisms, quantity	Antimycotic resistance profile	The relative number of poly resistant strains %
Candida albicans (n=40)	NCK□□I	22,5
	N□KF□□	15,0
	N□K□□I	15,0
	NC□FV□	12,5
	N□□F□□	12,5
	□□□□I	7,5
	□CKF□□	7,5
	□□□□□□	7,5
Candida parapsillosis (n=19)	N□□□□I	26,3
	□□□□□□	21,1
	N□K□□I	21,1
	□C□F□□	15,8
	NCKF□I	10,4
	NCKFVI	5,3
Candida tropicalis (n=17)	□□□□□□	29,4
	N□K□□I	23,4
	NCK□□ I	11,8
	N□□□□I	11,8
	□C□□□□	11,8
	NC□F□□	5,9
	NCKF□□	5,9
Candida stellatoidea (n=11)	N□K□□I	36,3
	NC□□□□	27,3
	□C□□□□	8,2
	□□□□□I	9,1
	□□□□□□	9,1
Candida glabrata (n=9)	N□K□□I	33,3
	N□□□□□	22,3
	□□□□□□	22,2
	NCK□□ I	11,1
	□□□F□□	11,1
Candida krusei (n=7)	N□KF□I	42,9
	N□□F□I	28,7
	□□□□□□	14,2
	□□KF□□	14,2

Despite the fact that in 27.3% of the observations, different types of bacterial microflora were isolated in these patients with otomycosis from the studied biolocus, *Candida* spp was isolated in the microflora. (C = 100%), their quantitative content was significantly higher ($p<0.05$) compared to the quantitative content of bacterial microflora, confirming the fact that *Candida* spp. this patient group is a leading etiologic agent. That is why it is necessary to consider the results of the sensitivity to the antimicrobials of each of the components of microbial associations. When choosing an antibiotic, it should be borne in mind that a number of antibiotics stimulate the rate of division of *Candida* cells, as some drugs can be a source of nitrogenous substances for these fungi.

While studying the results of the sensitivity of mold fungi isolated from patients with otomycosis in monoculture, we found that all strains of mold fungi of the genus *Aspergillus*, *Mucor* and *Penicillium* were resistant to nystatin (Table 3). 100% of *Penicillium* spp isolates. and *Mucor* spp. showed resistance to fluconazole and itraconazole. In addition, 86.4% of isolates of *Aspergillus* spp. showed resistance to clotrimazole and 68.2% to fluconazole.

DISCUSSION

In order to understand the trends in the formation of resistance to antifungal drugs fully, we have formed micromycetes resistance profiles that reflect a set of inherent and acquired resistance genes of a particular clonal population. Antimycotic resistance profiles are isolated markers that can be used for epidemiological analysis of pathogens and to determine patterns of their circulation. The formed resistance profile was thus NCKFVI, where N (nystatin), C (clotrimazole), K (ketoconazole), F (fluconazole), V (voriconazole), And (itraconazole). When interpreting a profile, a capital letter means a strain resistance to an antifungal or a moderate sensitivity, □ is the sensitivity of a microorganism to an antifungal drug. The data we obtained are presented in Table 3.

Among the strains of *Candida* spp. (n = 103), isolated from all patients with otomycosis, the percentages of antimycotics resistant to the study varied depending on the type of fungus and the presence of the associated bacterium. Thus, isolated strains of *Candida* spp. 17.5% were resistant to two types of antifungals; up to three – 32.0%; up to four – 20.4% of strains. It is noteworthy that 1.9% of *Candida* spp. were resistant to five and 1.0% to six types of antifungal agents, with these strains being attributed to *C. parapsilosis* after species identification and distinguished as a two-component fungal-bacterial association, *C. parapsilosis* + S. aureus.

10.7% of *Candida* spp. of the 32.0% who showed resistance to the three antifungals were isolated from the bioassay under study in the two- or three-component associations with *P. aeruginosa*.

The strains of *Candida* spp. studied by us with resistance to four antimycotics (20.4%) in 6.8% of cases they stood out in association with *K. pneumonia* as part of two and three-component fungal-bacterial associations. Besides, the analysis of the species spectrum of *Candida* fungi that showed resistance to three and four antimycotics showed that the greatest number of strains belonged to *C. albicans*: 14.6% of strains with 32.0% of resistant to three and 13.6% of 20.4% – resistant to four antimycotics.

The relationships of different microorganisms with yeast fungi are very diverse. In some cases, pronounced antagonism is manifested, in others the relationships are close to symbiotic, and sometimes similar to commensalism.

However, this is not so clear because, in particular, *Candida spp.* use mechanisms of coadhesion with bacteria of normal microflora for colonization of mucous membranes.

There is data on the direct influence of bacterial metabolism products on the process of attachment of cells to epitheliocytes through modification of adhesive molecules in the literature [7].

That is why it is essential to identify the interrelation between candidiasis and bacterial lesions with antibiotic therapy, with a mandatory study of the sensitivity of each of the association's microorganisms to antimicrobials and the abolition of ineffective antimicrobial therapy.

Fungal lesions of the external auditory canal are more common than not recognized, since with superficial lesions they are of little concern to the patient. Only when the mycelium germinates into the depth of the skin, symptoms of its irritation occur: itching, increased desquamation of the epidermis, minor serous secretions, walls of the auditory canal swollen, hyperemic, painful [8].

In patients with otomycosis, the etiological agent of which was the fungal fungi of the genus *Aspergillus* (16.7%) and *Penicillium* (4.6%), the quantitative content of the microbiota was in the diagnostic range (≥ 105 CFU / ml swab wash).

Among all species of fungi of the genus *Aspergillus* (n = 22), 77.3% of the strains were susceptible to Voriconazole; 63.6% to Ketoconazole; 36.4% – to Itraconazol; 31.8% to Fluconazole and 13.6% to Clotrimazole. Isolate Penicillium spp. sensitive to Voriconazole (100%); Ketoconazole (83.3%) and Clotrimazole (50.0%).

The obtained resistance profiles of isolated molds are presented in table IV.

Analyzing the micromycetes resistance profiles obtained, it is proved that there is a high probability of resistance of isolates within the genus and species. In addition, there is a high likelihood of fluctuation in the results of antimicrobial resistance in different regions. All this leads to the need to take into account the individual and regional peculiarities of the species composition of pathogens, the study of their resistance, including to antifungal drugs.

The analysis of modern literature contains data on the level of sensitivity of microorganisms to certain antimicrobials, although in different countries, regions and even medical institutions the sensitivity of microorganisms to antibiotics varies greatly. Therefore, systematic monitoring of the species composition of the microbiota and its sensitivity to antimicrobials should be made at all levels – international, national, regional and local.

In addition, a personalized approach to diagnosis, timely diagnosis and the search for effective treatments will help the patient recover quickly and prevent complications.

Table 4. Resistance profiles of isolated fungi of the genus Aspergillus and Penicillium.

Kind of microorganisms, quantity	Antimycotic -resistance profile	The relative number of strains, %
Aspergillus niger (n=17)	NC$_{\square}$F$_{\square}$I	41,2
	NCK$_{\square\square}$I	29,4
	NC$_{\square}$FV$_{\square}$	17,6
	N$_{\square\square}$F$_{\square}$I	11,8
Aspergillus flavus (n=3)	NC$_{\square\square\square}$I	66,7
	N$_{\square}$K$_{\square\square\square}$	33,3
Aspergillus fumigatus (n=2)	NCK$_{\square\square\square}$	100
Penicillium notatum (n=3)	NC$_{\square}$F$_{\square}$I	66,7
	N$_{\square\square}$F$_{\square}$I	33,3
Penicillium chrysogenum (n=3)	N$_{\square}$KF$_{\square}$I	66,7
	NCKF$_{\square}$I	33,3

CONCLUSIONS

The general structure of the species spectrum of etiologically relevant agents of otomycosis: *Candida spp.* (78.0%) of the total isolate spectrum, with 65.0% of *Candida* strains isolated in monoculture and 35.0% in bacterial association; fungi of *Aspergillus spp.* (16.7%); *Penicillium spp.* (4.6%) and *Mucor spp.* (0.7%).

Among fungal-bacterial associations (27.3%) the only representative of mycobiota were found *Candida spp.* (C = 100%); the total degree of insemination *Candida spp.* was significantly (p <0.05) higher than the rate of bacterial bacterial contamination.

The sensitivity of isolated micromycetes to antifungals varied between different types of fungi and between different antifungals. The highest antifungal resistance was shown by the strains of isolated yeast and fungi against nystatin. 1.0% of *C. parapsilosis* strains were resistant to six types of antifungal agents, with these strains being distinguished as part of a two-component fungal-stokococcal association.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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