ALLERGOLOGY

Yeast Fungi in Patients with Allergic Diseases: Species Variety and Sensitivity to Antifungal Drugs

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Yeast microflora was studied in the skin of 91 patients with atopic dermatitis, in bronchial secretion of 13 patients with bronchial asthma and 8 patients with allergic bronchopulmonary mycosis. Forty-eight isolates were obtained. Alypophilic yeast fungi isolated from the skin were presented mainly by genera *Candida spp.* (48%) and *Rhodotorula spp.* (29%), while the cultures isolated from bronchial secretion mainly by *Candida albicans.* The sensitivity of yeast cultures to the antifungal drugs diflucan, clotrimazole, nizoral, orungal, exoderil, levorin, pymafuzin, and nitrofungin was determined. The most efficient drugs were diflucan, clotrimazole, nizoral, and orungal. More than half isolates were sensitive only to the high concentrations of levorin (48%), pymafuzin (75%), and nitrofungin (82%); 64% isolates were insensitive even to high concentrations of exoderil. Preliminary *in vitro* selection of the antifungal drugs is required for efficient elimination of the yeasts.

Key Words: *yeasts; fungi; antifungal drugs; sensitivity; atopic dermatitis; bronchial asthma; bronchomycosis*

The last decade significantly widened the range of clinically important yeasts. At present, 18 genera [5] compared to previous 7 ones [8] were detected in the patients with superficial and deep mycoses. Previously we showed that yeast alypophilic fungi present on the skin of 40% patients with atopic dermatitis (AD) drastically aggravate the course of the disease [1]. The generic composition of yeast microflora in patients with AD was determined: 53% isolates belonged to genus *Candida*, 21% to *Rhodotorula spp.*, 5% to *Cryptococcus spp.*, and 3% to *Trichosporon spp.* In healthy individuals the yeasts were found in 3% cases only, dissemination being 0-20 CFU/dm^2 [1]. Elimination of yeasts with antifungal drugs considerably promoted healing of skin lesions [2]. However, in some cases choosing the adequate preparation *in vivo* takes long time and is very expensive. In light of this, rapid identification and *in vitro* determination of sensitivity of isolated cultures to antifugal drugs is of crucial importance.

Our aim was to study species composition and sensitivity to antifungal drugs of the yeast fungi isolated from patients with allergic diseases: AD, bronchial asthma (BA), and allergic bronchopulmonary mycosis (ABPM).

MATERIALS AND METHODS

Patients hospitalized in I. I. Mechnikov Allergological Center in 1998-99 were examined. Group 1 (n=91) comprised patients with AD at the age of 3 months to 36 years. Group 2 (n=21) comprised patients at the age of 6-65 years with BA (n=13) and acute ABPM (n=8).

Specimens from affected skin of patients with AD were transferred to bacterial containers (Lenmedpolymer) with modified Sabouraud's medium containing

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test antibiotics [1]. Bronchial secretion (sputum) from patients with BA and ABPM was inoculated to Petri dishes with the same medium. The dishes and bacterial containers were incubated at 25-30°C.

Monotypic colonies were reinoculated on Sabouraud's agar slants and identified using cultural, morphological, cytological, and physicobiochemical tests (a total of 37-50 indices [13]). Specific identification was performed by the method of Barnett with assessment of the obtained parameters with "Yeast Identification Program.4" software. The probability of identification was determined and specified for each culture. The probability of identification from 0.7 to 0.9 was considered acceptable (maximum value is 1). Probability of identification decreased in cases, when the program assigned the yeasts to different serotypes within the same species with equal probability, for example to *Cryptococcus albidus var. albidus* and *Cr. albidus var. aerius*; *Candida albicans var. albicans* and *C. albicans var. stellatoidea.*

Sensitivity of isolates to antifungal drugs diflucan, clotrimazole, nizoral, orungal, exoderil, levorin, pymafuzin, and nitrofungin was assessed during 3 days by the microdilution method [6,11]. The concentration of *Candida* yeasts in the final inoculate was 1×10^3 CFU/ml, and the concentrations of *Rhodotorula* and other yeasts were 1×10^4 CFU/ml. The low concentrations of the drugs in the final suspension were 0.5-2.0 μg/ml, while the high concentrations were 4-20 μg/ml. In respect to a particular drug all yeast fungi were subdivided into 3 categories: sensitive isolates that did not grow in the presence of low and high drug concentrations (I); moderately sensitive isolates that grew at low, but not at high drug concentrations (II), and insensitive isolates that grew both at low and high drug concentrations.

TABLE 1. Yeast Fungi from Skin of Patients with Atopic Dermatitis

Genus		Probability of identification	Localization	Dissemination, CFU/dm²	Age, years
Candida	*albicans*	0.288	Mouth edges	352	1.5
	albicans	0.857	Cheeks	66	2.5
	apis	0.979	Cheeks	1408	2
	azyma	0.967	Buttocks	88	3
	blankii	0.412	Hands	220	0.2
	bombicola	0.909	Face, ulnar flexure	264; lawn	12
	bombicola	0.492	Hands	198	2.5
	bombicola	0.891	Hands	308	24
	haemulonii	0.909	Mouth edges	484	2
	terebra	0.792	Face	1452	0.7
	versatilis	0.908	Scalp	Lawn	35
	versatilis	0.402	Mouth edges	66	12
	versatilis	0.549	Face	1210	11
Rhodotorula	*aurantiaca*	0.963	Axilla	66	3.5
	aurantiaca	0.982	Hands	66	12
	aurantiaca	0.957	Cheeks	110	2.5
	aurantiaca	0.975	Face	198	0.25
	aurantiaca	0.986	Hands	66	3
	glutinis	0.975	Cheeks	66	1.25
	minuta	0.609	Cheeks	88	2.5
	mucilaginosa	0.981	Cheeks	242	
Cryptococcus	*albidus*	0.492	Abdomen	198	2
	albidus	0.473	Scalp	264	5
Debaryomyces	*hansenii*	0.478	Cheeks	220	1
	hansenii	0.951	Mouth edges	88	6
	polymorphus	0.325	Cheeks	110	1.75
Trichosporon	*ovoides*	0.940	Face, hands	440; 176	2.5

TABLE 2. Yeast Fungi in Bronchial Secretion of Patients with Allergic Bronchopulmonary Diseases

Genus		Patient's age	Diagnosis	Probability of identification	Degree of dissemination, CFU/ml
Candida	*albicans*	43	ABPM	0.875	30
	albicans	29	ABPM	0.912	2900
	albicans	32	BA	0.846	220
	albicans	57	ABPM	0.930	160
	albicans	6	ABPM	0.378	100
	albicans	61	ABPM	0.541	2200
	albicans	64	BA	0.312	50
	albicans	18	BA	0.546	100
	albicans	35	BA	0.972	650
	albicans	58	ABPM	0.154	2500
	glabrata	52	ABPM	0.311	825
	haemulonii	37	BA	0.975	100
	haemulonii	65	ABPM	0.875	600
	haemulonii	52	ABPM	0.976	250
	versatilis	52	ABPM	0.530	300
	zeylanoides	8	BA	0.884	100
Rhodotorula	*glutinis*	32	BA	0.878	20
	rubra	57	ABPM	0.945	20
Kluyveromyces	*marxianus*	63	BA	0.896	250
	marxianus	63	BA	0.978	63
Saccharomyces	*bayanus*	33	BA	0.277	600

RESULTS

In 37 (41%) patients with AD, yeasts were found in affected skin, which agrees with previous data [1]. In 25 (27%) patients yeast dissemination exceeded 40 CFU/dm^2; in 16 patients (18%) including 10 children below 2 years dissemination was 200 CFU/dm^2 and in 4 (4.4%) patients it was 1000 CFU/dm^2.

Twenty-seven isolates were obtained from 25 patients, in whom yeast dissemination surpassed 40 CFU/dm^2 (2 patients had two yeast cultures of different genera, Table 1). In AD patients inoculations performed from 2-3 affected skin sites revealed high incidence of yeast on facial skin (in 63% cases). According to W. K. Noble [3], the face is favorite ecological niche of resident microflora in humans. Therefore, detection of yeast fungi on the face of AD patients attests to the chronic character of the disease and indicates necessity to eliminate the adverse component of ecological system.

Twenty-seven isolates were distributed as follows: *Candida* (48%), *Rhodotorula* (29%), *Cryptococcus* (7.4%), *Debaryomyces* (11%), and *Trichosporon* (3.7%).

The isolates of *Candida* genus were assigned to the following species: *C. albicans, C. apis, C. azyma, C. bombicola, C. haemulonii, C. terebra,* and *C. versatilis*. The isolates of *Rhodotorula* genus were presented by *R. aurantiaca, R. glutinis, R. minuta,* and *mucilaginosa* species. Genera *Cryptococcus* and *Trichosporon* were presented by species *Cr. albidus* and *T. ovoides*, respectively. The isolated *Debaryomyces* included species *D. hansenii* and *D. polymorphus*.

We found no published data on species variety of yeast fungi in patients with bronchopulmonary allergic diseases. There is evidence on high incidence (27-90% cases) of *Candida* yeast in the respiratory tract of the patients with BA [4,10,12], although taxonomy of the isolates was not specified in these papers, or all culture were assigned to *Candida albicans*.

In 21 patients, bronchial secretion was inoculated to detect microflora. In one patient with BA no fungi were found, in 4 patients the yeast were found with mould fungi of *Aspergillus* genus, and only *Aspergillus* or only yeast were found in 3 and 12 patients, respectively. In total, 21 yeast isolates were obtained (in 4 patients 2-3 different cultures were found).

Most isolated cultures (76%) were presented by *Candida* genus, in which *C. albicans* prevailed (Table 2). Some yeast genera were relatively rare: genus *Rhodotorula* (9.5% cases) was presented by species *R.*

glutinus, and *R. rubra*, while *Kluyveromyces marxianus* and *Saccharomyces bayanus* were observed in 9.5 and 4.8% cases. A high degree of dissemination in the bronchial secretion (above 300 CFU/ml) was most characteristic of *Candida* yeast, and its incidence was 1.7-fold greater in patients with ABPM than with BA.

Since elimination of yeast fungi is of considerable importance for the treatment of allergic skin and respiratory diseases, it is important to assess the sensitivity of isolated cultures to antifungal drugs.

Diflucan, clotrimazole, nizoral, and orungal most efficiently suppressed yeast growth (Fig. 1). The percentage of cultures highly sensitive to other drugs was comparatively low (8-19%), although many isolates were sensitive to high concentrations of nitrofungin, pymafuzin, and levorin. Most cultures (64%) were completely insensitive to exoderil.

It should be noted that some strains of yeast fungi were insensitive even to highly efficient drugs diflucan, clotrimazole, nizoral, and orungal, while 3% isolates were insensitive to nitrofungin and 6% to pymafuzin. Such strains were found among all isolated yeast genera, although most of them belonged to *Candida* genus. Hence, *in vitro* evaluation of sensitivity to antifungal drugs is of great importance for efficient elimination of yeast fungi.

It will be reasonable to compare sensitivity of various yeast genera to a number of antifungal drugs in future analysis of larger number of isolates.

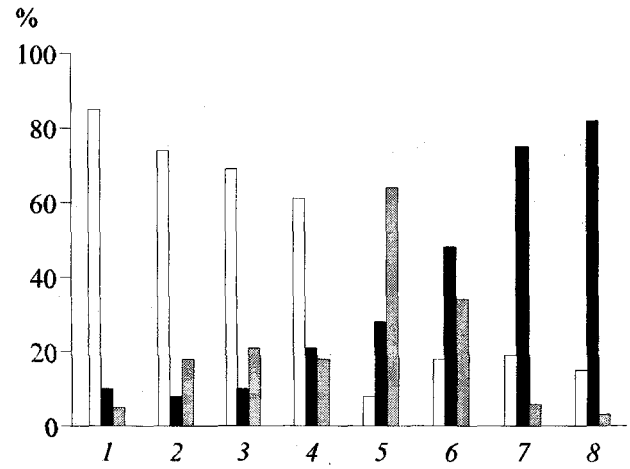

Fig. 1. Efficiency of antifungal drugs in cultures of yeast fungi. Ordinate: % of total number of tested cultures. *1*) diflucan, *2*) clotrimazole, *3*) nizoral, *4*) orungal, *5*) exoderil, *6*) levorin, *7*) pymafuzin, and *8*) nitrofungin. Open, solid, and hatched bars correspond to I, II, and III degree of sensitivity, respectively.

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