DISTRIBUTION AND SUSCEPTIBILITY OF *CANDIDA* SPECIES ISOLATED IN THE MEDICAL UNIVERSITY OF DEBRECEN

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Data of *Candida albicans* and non-albicans *Candida* species isolated during the 1997–2000 period in the Medical and Health Science Center of the University of Debrecen are analysed. The number of yeast isolates increased from 408 to 1213 per year during this period. Dominance of *C. albicans* has been persistent, but a slight increase of *C. glabrata* and *C. krusei* could be observed. Distribution of different *Candida* species isolated from 16 body sites indicates that *C. albicans* seems to be still the most aggressive *Candida* species. Investigation of 244 urinary *Candida* isolates (parallel with bacterial cultures) suggests that tha aetiological role of *Candida* species in the pathogenesis of urinary tract infections can be hypothesized if colony forming unit (CFU) number of yeasts is higher than 10^4/ml and bacteria are present in low CFU number or are absent. Antifungal susceptibility testing of *C. albicans, C. glabrata, C. tropicalis* and *C. krusei* against Flucytosine, Amphotericin-B, Miconazole, Ketoconazole and Fluconazole suggests that Amphotericin-B is still the most effective antifungal agent. Finally, the problems in judging the aetiological role of isolated *Candida* species in the pathogenesis of different types of diseases are critically discussed.

Introduction

The number of human infections caused by yeasts has been increased dramatically. This fact is attributed to several factors, e.g. growing number of immunocompromised patients [1, 2], incorrect use of antibiotics, etc. On the other hand, development of laboratory techniques used in medical mycology, which allow isolation and identification more and more yeast species, may also be responsible for this alarming increase.

Based on the abovementioned tendencies, first aim of our study was to demonstrate the results of identification of yeast strains isolated in our laboratory, with special respect to the relationship between culture results and disease. The study tries to explore the possible causes of false positive results, e.g. the contaminations. Finally, we summarize our data on antimycotic resistance of the most frequently isolated *Candida* species.

Our data are based on culture results of our laboratory from 1997 to 2000. During this period the number of yeast isolates increased from 408 to 1213 per year. In order to underline the importance of precise identification of pathogenic *Candida* species and determination of antimycotic susceptibility we also present some cases, where the correct identification of the causative *Candida* species was critical.

Materials and methods

Specimens from non-sterile sites were cultured for yeasts if requested by the physician, and were plated directly onto Sabouraud dextrose (SD) agar. Specimens from sterile sites were routinely inoculated into both SD broth and agar media. We determined the CFU number/ml in midstream urine samples by means of Uricult Plus (Orion Diagnostica) and in sputum and bronchial specimens by means of quantitative culture.

Identification of yeasts grown on the primary SD agar plate was performed by the germ tube test [3] and CHROMagar Candida (Beckton Dickinson) medium. In doubtful cases or when the isolate could not be identified with the above methods, a biochemical panel (API ID32C, BioMerieux) was used. In some selected cases we used pulsed-field gel electrophoresis (PFGE) for species identification.

Antifungal sensitivity of the identified isolates was tested against the most frequently used six drugs by breakpoint determination (Fungitest, Sanofi Pasteur). This method was quality-controlled with *C. albicans* ATCC 90028, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 using E-test (AB Biodisk). Itraconazole susceptibilities were not evaluated due to its inaccurate breakpoint.

Determination of minimal inhibitory concentration (MIC) of Fluconazole, Itraconazole and Amphotericin-B was performed using E-test (AB Biodisk) in selected cases. Results were evaluated according to NCCLS standards.

Results

Distribution of the five most frequent *Candida* species isolated in our laboratory during the 1997–2000 period is shown in Table I. In addition to the increasing number of the isolations the increase of *C. glabrata* seems to be a significant phenomenon (9.3% in 1997 and 17.4% in 1999) in spite of the fact that in 2000 a decrease of isolation percentage could be observed. A slight, but similar tendency was observed in the case of *C. krusei* (2.7% in 1997 and 4.3% in 2000). *C. albicans* is still the most frequently isolated yeast species, above 60% during the four years of the study.

Table II shows the distribution of yeast isolates in 2000. Multiple isolates from the same sample site of the same patient are considered as one isolation.

Fungaemia was detected 29 times from twelve patients. Predisposing factors were low birth weight (two cases), pancreatitis (five cases) and serious surgical interventions (five cases). Though the majority of fungaemias were caused by *C. albicans*, the more infrequent non-albicans fungaemias are also important. To prove this here we briefly present two cases. In the first case, polyresistant *C. krusei* was isolated from haemoculture of a girl suffering from acute myelogenous leukemia (AML). Besides the one positive haemoculture, *C. krusei* was repeatedly isolated from her urine and wound samples. The other case was a *C. parapsilosis* endocarditis (10 positive haemocultures and 2 positive valve samples) in a previously healthy male patient.

Out of 23 patients with tracheal cultures positive for yeasts, in two cases the same fungus was isolated from their sputum. Similarly, sputa of two of the 29 patients whose bronchial fluid samples contained *Candida* species, have also proved to be positive for the same species. The tracheal culture of a patient was positive for *C. albicans* three weeks after the first isolation. Seventy-five per cent of yeasts cultured from lower respiratory tract samples were *C. albicans*.

Aetiologic role of yeasts isolated from sputum was evaluated if the yeasts were present in high colony forming unit (CFU) number and/or pure culture (76 cases). Clinical findings indicated that *C. albicans* might have been responsible for symptoms in 19 cases, *C. glabrata* in four cases, *C. tropicalis, C. krusei* and *C. kefyr* in one case each. *C. albicans* was repeatedly cultured from sputum of two patients within two months. Sputa of three patients remained *C. albicans* positive for a three months

period. Similarly, *C. tropicalis* was found to be present in a sputum sample of a patient several weeks after the initial isolation of the yeast from sputum.

We isolated *Candida* species from upper respiratory tract specimens very frequently, mainly from samples of pediatric patients. In addition to the large number of patients with yeast positive samples (361) 15 of these patients were repeatedly positive.

Twenty-six ear samples were yeast positive. Eleven specimens from pediatric intensive care units proved to be positive for *C. albicans*. In one case wound culture of the newborn was also positive for the same species.

Relatively high number of wound cultures (91) proved to be positive for *Candida* species. "Superficial" wound specimens were obtained from burned patients. "Deep" wound specimens were obtained mainly from patients suffering from different types of malignancies. Their haemoculture results were positive for the same Candida species only in three cases.

Concerning bile specimens we report only those with pure Candida cultures. Twenty-nine samples of 16 patients proved to be positive for *C. albicans*. Similarly, we have obtained pure cultures of *C. glabrata* (5 samples, 4 patients), *C. kefyr* (3 samples, 4 patients), *C. krusei* (2 samples, 3 patients) and one sample of *C. parapsilosis* and of *C. famata*.

Similar principles were followed in case of vaginal candidiasis (106 isolations). *C. albicans* and *C. glabrata* cultures were obtained from 24 and 3 patients, respectively. *C. albicans* was repeatedly isolated from vaginal samples of 5 patients. *C. glabrata* could be cultured repeatedly from vagina only in one case.

Seventy-eight yeast positive urine cultures of 55 male patients was tested. Urine samples of 8 males proved to be repeatedly positive for yeasts within 48 hours (the same species has been found in similar CFU number). 166 samples of 135 women were positive for yeasts. Repeated sampling within 48 hours yielded the same species in similar CFU number only in eleven cases.

The number of positive urine samples, possible contaminations (taking into account the CFU numbers of different microbes) and the distribution of male and female infections are comparatively demonstrated on Table II and Table III Data show that in urine samples obtained from patients with single candida infections the CFU number was at least 10^4/ml in 44 and 135 males and females, respectively. The number of multiple (fungal and bacterial) infections is low (45) and in these cases a fungal dominance could be observed. An interesting additional difference between the two patient groups is the higher prevalence of *C. tropicalis* and *C. parapsilosis* in the male group and that of *C. glabrata* and *C. krusei* in the female group.

Tables IV, V, VI and VII summarize the antimycotic resistance of *Candida* strains isolated in 2000. The resistance of *C. albicans* against the tested antimycotic drugs was low. Seventeen isolates proved to be intermediate to Amphotericin-B, while being sensitive to Fluconazole. Flucytosine and Amphotericin-B were similarly effective against *C. glabrata* (97.8% and 96.7%, respectively), while azole-type drugs were less effective. Ninety-one per cent of *C. tropicalis* isolates proved to be sensitive to Fluconazole, resistance proved to be relatively high in case of other tested antifungal drugs. Seventy-eight per cent of *C. krusei* strains were sensitive, 22% were moderately sensitive to Amphotericin-B. Resistant isolate was not found. *C. parapsilosis* isolates were highly sensitive to all tested antimycotic drugs, except for miconazole.

Discussion

It is not easy to judge the aetiological role of *Candida* species in the pathogenesis of certain diseases. Each case requires individual judgement of possible aetiological relationships. Correct judgement has to be based on team work of the physician, the infectologist and the clinical microbiologist, sometimes involving a clinical hygienist as well [4].

The considerable increase of *Candida* isolations by our laboratory during the past four years (Table I) is based on at least three facts. First, the development of mycological laboratory techniques reveals more fungal pathogens, which were overlooked in the past. Practice of laboratory routine has also changed (routine use of fungal media, different and longer incubation temperatures etc.). A diagnostic algorithm has been developed in our laboratory [5].

Secondly, the well-documented accumulation of immunocompromised patients also contributes to the increasing frequency of yeast isolations [6, 7], raising the necessity of molecular epidemiological studies on yeasts. PFGE is a suitable method for determination of identity of isolated strains [8, 9].

The third reason of increase in *Candida* isolations is the increasing demand for fungal cultures.

It is well known that the background of candidaemias is always a serious disease, frequently some sort of immunosuppression. From therapeutical point of view the outcome of infection largely depends on the antifungal resistance of the fungus. *C. krusei*, being intrinsically resistant to fluconazole, represents a serious problem [2, 10]. In infections caused by *C. krusei* Amphotericin-B is the drug of choice. Patients suffering from AML mentioned above were successfully treated with this drug. However, the struggle with the fungus was not easy. Three weeks after initiation of Amphotericin-B therapy the previously susceptible *C. krusei* became moderately

susceptible and only administration of liposomal Amphotericin-B for six weeks was capable to eradicate the fungus.

Evaluation of results of fungal cultures from non-sterile body sites requires caution. In case of sputum and urine, pure culture and repeated isolations of the same species support the aetiological relationship. With the exception of one case, the eleven yeast positive ear specimens of newborns (which were obtained within few days after birth) can be considered a result of colonization from the vagina of the mother. In the excepted case the same species was isolated from other body sites as well, supporting the possibility of pathogenic role of the isolated yeast.

The non-life threatening but very unpleasant vulvovaginitis also has special mycological aspects [11, 12]. Yeast isolates from patients suffering from recurrent vulvovaginitis frequently show different karyotypes [13].

Data of Table III raises the possibility of aetiological role of fungi in urinary tract infection if a urine culture contains at least 10^4 CFU/ml yeasts and no or very low number of bacteria. Aetiological role of cultured yeasts is supported by the repeated positivity of samples obtained within 48 hours. However, the evaluation of candidurias is very difficult and requires further studies [14, 15].

Two important conclusions can be drawn from studies on antimycotic sensitivity of isolated fungi (Tables IV, V, VI, VII). Primary antifungal resistance of some species to antimycotic drugs, mainly to azole derives has clearly been demonstrated [2]. However, the increasing tendency of development of antimycotic resistance necessitates the determination of resistance pattern of isolated species. Antimycotic sensitivity testing has several technical problems [16, 17]. Results may depend on the method used and not all methods are capable of detecting primary resistance. Preliminary studies of our laboratory [5] indicate the importance of such investigations.

As a conclusion, there is no doubt that an increasing role of *C. albicans* and the non-albicans *Candida* species can be observed in many human diseases. In this report we have tried to underline the importance of correct interpretation of culture results. Finally, two aspects of future perspectives should be mentioned here. Collection of sequentially isolated *Candida* strains is very useful in studies of pathogenesis of yeast infections, since a possible explanation of the increase of candida-related diseases may be the selection of more virulent *Candida* strains. Regarding the changing resistance patterns, another conclusion of this study is the need for searching for new antifungal drugs acting on new targets [18].

Table I

Species distribution of Candida isolates

Species	Year							
	1997		1998		1999		2000	
	№	%	№	%	№	%	№	%
C. albicans	257	62.99	388	65.65	507	66.54	803	66.19
C. glabrata	38	9.31	78	13.19	133	17.41	138	11.37
C. tropicalis	20	4.90	25	4.23	27	3.5	61	5.03
C. krusei	11	2.70	20	3,38	30	3.9	52	4.30
C. parapsilosis	6	1.47	9	1.52	13	1.7	32	3.21
Others	76	18.60	71	12.00	53	6.95	120	8.89
Altogether	408	100.00	591	100.00	763	100.00	1206	100.00

Table II

Sampling site distribution of yeast species in 2000

	C. albicans	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. parapsilosis*	*C. kefyr*	other *Candida* species	unidentified yeast	altogether
haemoculture	7	0	2	1	1	0	0	1	12
heart valve	0	0	0	0	1	0	0	0	1
iv. catheter	7	1	1	0	0	0	1	1	11
bronchus	21	3	2	1	0	1	0	1	29
tracheal tube	14	4	1	0	1	0	1	2	23
sputum	49	9	2	2	2	5	2	3	74
upper airways	270	15	17	13	4	9	3	24	355
coniunctiva	2	0	0	0	0	0	0	0	2
ear	13	1	0	0	6	0	0	5	25
wound	37	20	10	3	6	1	6	8	91
nail	4	2	1	0	0	0	2	1	10
bile	29	9	2	3	2	3	2	4	54
vagina	89	9	1	0	0	0	2	5	106
urethra	5	1	0	0	0	0	0	0	6
sperm	2	0	0	0	0	0	0	0	2
urine	119	30	7	12	4	4	3	7	186
altogether	668	104	46	35	27	23	22	62	987

Table III

Species distribution of yeasts isolated from urine in 2000. CFU: colony forming unit

Species	Yeasts isolated in pure culture							
	men				women			
	CFU				CFU			
	10^5/ml	10^4/ml	10^3/ml	10^2/ml	10^5/ml	10^4/ml	10^3/ml	10^2/ml
C. albicans	16	8	7	3	35	27	18	4
C. glabrata	3	3	0	0	18	5	3	2
C. tropicalis	8	0	1	8	0	1	0	0
C. parapsilosis	1	3	1	0	0	0	0	0
C. krusei	2	0	0	0	8	6	1	1
C. kefyr	0	0	0	0	3	0	1	0
C. sake	0	0	0	0	2	0	0	0
Altogether	30	14	9	11	66	39	23	7

Species	Yeasts isolated together with bacteria							
	Men				women			
	CFU				CFU			
	10^5/ml	10^4/ml	10^3/ml	10^2/ml	10^5/ml	10^4/ml	10^3/ml	10^2/ml
C. albicans	3	3	3	3	3	6	1	4
C. glabrata	1	0	0	0	3	3	2	1
C. krusei	0	0	0	0	0	4	3	0
C. tropicalis	0	0	0	0	1	0	0	0
Candida spp.	0	1	0	0	0	0	0	0
Altogether	4	4	3	3	7	13	6	5

Table IV

Susceptibility data of *Candida albicans* isolates in year 2000
S: susceptible; I/D: intermediate/dose-dependent susceptible; R: resistant

Antifungal drug	*C. albicans*					
	S		I/D		R	
	№	%	№	%	№	%
Flucytosine	407	98.1	4	1.0	4	1.0
Amphotericin-B	398	95.9	17	4.1	0	0.0
Miconazole	400	96.4	15	3.6	0	0.0
Ketoconazole	380	92.5	16	3.9	15	3.6
Fluconazole	532	96.0	14	2.5	8	1.4

Table V

Susceptibility data of *Candida glabrata* isolates in year 2000
S: susceptible; I/D: intermediate/dose-dependent susceptible; R: resistant

Antifungal drug	*C. glabrata*					
	S		I/D		R	
	№	%	№	%	№	%
Flucytosine	87	97.8	0	0.0	2	2.2
Amphotericin-B	87	96.7	2	2.2	1	1.1
Miconazole	76	85.4	13	14.6	0	0.0
Ketoconazole	66	74.2	23	25.8	0	0.0
Fluconazole	93	83.0	19	17.0	0	0.0

Table VI

Susceptibility data of *Candida tropicalis* isolates in year 2000
S: susceptible; I/D: intermediate/dose-dependent susceptible; R: resistant

Antifungal drug	*C. tropicalis*					
	S		I/D		R	
	№	%	№	%	№	%
Flucytosine	28	71.8	2	5.1	9	23.1
Amphotericin-B	34	79.1	8	18.6	1	2.3
Miconazole	7	17.9	32	82.1	0	0.0
Ketoconazole	14	35.9	22	56.4	3	7.7
Fluconazole	48	90.6	2	3.8	3	5.7

Table VII

Susceptibility data of *Candida krusei* isolates in year 2000
S: susceptible; I/D: intermediate/dose-dependent susceptible; R: resistant

Antifungal drug	*C. krusei*					
	S		I/D		R	
	№	%	№	%	№	%
Flucytosine	2	4.7	39	90.7	2	4.7
Amphotericin-B	35	77.8	10	22.2	0	0.0
Miconazole	2	4.7	40	93.0	1	2.3
Ketoconazole	4	9.3	36	83.7	3	7.0
Fluconazole	0	0.0	40	85.1	7	14.9

References

1. D'Antonio,D., Iacone,A., Schioppa,F.S., Bonfini,T., Romano,F.: Effect of the current antimicrobial therapeutic strategy on fungal colonisation in patient with hematologic malignancies. Curr Microbiol **33**, 118 (1996).

2. White,T.C., Marr,K.A., Bowden,L.A.: Clinical, cellular and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev **11**, 382 (1998).

3. Merz,W.G., Roberts,G.D.: Detection and recovery of fungi from clinical specimens. in: Balows,A. (ed.): Manual of clinical microbiology. American Society for Microbiology, Washington D.C. 1991

4. Mestyán,Gy.: The consulting clinical microbiologist. (in Hungarian) Infektológia és klinikai mikrobiológia **5**, 124 (1998).

5. Szabó,B., Majoros,L., Miszti,C.: Microbiological diagnosis of fungal infection. in: Maródi,L. (ed.): Emerging infectious diseases in Eastern-Central Europe. Medicina Budapest, 2000

6. Vincent,J.L., Anaisie,E., Bruining,H., Demajo,W., El-Ebiary,M., Haber,J., Hiramatsu,Y., Nitemberg,G., Nyström,P.O., Pittet,D., Rogers,T., Sandven,P., Sganga,G., Schaller,M-D., Solomkin,J.: Epidemiology, diagnosis and treatment of systemic Candida infection in surgical patients under intensive care. Intensive Care Med **24**, 206 (1998).

7. Waggoner-Fountain,L.A., Walker,M.W., Hollis,R.J., Pfaller,M.A., Ferguson,J.E., Wenzel,R.P., Donowitz,L.G.: Vertical and horizontal transmission of unique Candida species to premature newborns. Clin Infect Dis **22**, 803 (1996).

8. Fodor,E., Bácskay,T., Nagy,A., Nagy,E.: Karyotyping of human pathogen Candida species by pulsed-field gel electrophoresis. (in Hungarian) Klin Kisérl Lab Med **24**, 179 (1997).

9. Iwaguchi,S., Homma,M, Tanaka,K.: Variation in the electrophoretic karyotype analysed by the assignment of DNA probes in Candida albicans. J Gen Microbiol **136**, 2433 (1990).

10. Samaranayake,Y.H., Samaranayake,L.P.: Candida krusei: biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. J Med Microbiol **41**, 295 (1994).

11. Sobel,J.D., Chaim,W.: Vaginal microbiology of women with acute recurrent vulvovaginal candidiasis. J Clin Microbiol **34**, 2497 (1996).

12. Fidel,P.L.Jr., Cutright,J., Steele,C.: Effects of reproductive hormones on experimental vaginal candidiasis. Infect Immun **68**, 651 (2000).

13. Majoros,L., Miszti,C., Szabó,B.: Isolation and identification of Candida albicans and non-albicans species from humans by traditional and new methods. (abstract) 13th International Congress of the Hungarian Society for Microbiology, Budapest, 1999

14. Sobel,J.D., Vazquez,J.A.: Fungal infections of the urinary tract. World J Urol **17**, 410 (1999).

15. Sobel,J.D., Kauffman,C.A., McKinsey,D., Zervos,M., Vazquez,J.A., Karchmer,A.W., Lee,J., Thomas,C., Panzer,H., Dismukes,W.E. and the National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group: Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. Clin Infect Dis **30**, 19 (2000).

16. Dósa,E., Nagy,E.: Evaluation of identification and antifungal susceptibility testing of clinical isolates of candida species. (in Hungarian) Klin Kisérl Lab Med **24**, 189 (1997).

17. Barcs,I., Bálint,E., Bajó,G.: Antifungal resistance of strains of clinical origin. (in Hungarian) Infektológia és klinikai mikrobiológia **8**, 25–33 (2001).

18. Pócsi,I., Sámi,L., Leiter,É., Majoros,L., Szabó,B., Emri,T., Pusztahelyi,T.: Searching for new type antifungal drugs. Acta Microbiol et Immunol **48**, 533 (2001).