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Incidence of Candida Tropicalis in Clinical Samples over 5-year Period

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Abstract: *C.tropicalis* strains were identified by cultivation on Sabouraud glucose agar, CHROMagar Candida, filamentous test, and the ability to assimilate and ferment saccharides. *C.tropicalis* was isolated from adult patients in 38 cases (63.3%) and from children in 22 cases (36.7%). The highest recovery of this fungus was recorded for colpitis (n=8) and in premature babies (n=7) admitted to neonatal intensive care unit. High incidence of isolation of *C.tropicalis* was recorded for gynecological material (n=20) and the oral cavity (n=15).

Key words: Candida species, *Candida tropicalis*, fungal colpitis, praematurity Incidence of *Candida tropicalis*

Introduction

Systemic candidosis is a frequent complication in intensive immunosuppressive therapy, total parenteral feeding, abdominal post-surgical interventions, and long-term wide-spectrum antibiotic treatment. Most of Candida infections are induced by most frequently occuring fungus microorganism, *Candida albicans*. Mycological studies of a number of studies point to increasing importance of *C.tropicalis* as a pathogen occuring in various diseases in humans (Al-Hedaithy and Fotedar, 1997; Faure-Fontenla, *et al.*, 1997; Shmuely; *et al.*, 1997; Weers-Pothoff, *et al.*, 1997, Gerritsen, *et al.*, 1998).

C.tropicalis was described as the main cause of candidoses induced by species of non-albicans Candida and was isolated from the clinical material obtained from oncological patients in 48% of cases (Wingard; 1996; Abi-Said; *et al.*, 1997; Nucci *et al.*, 1998). This yeast was frequently found in samples from oral cavity of HIV-positive patients showing signs of oropharyngeal candidosis (in 18 %) and in women suffering from vulvovaginitis (in 6%) (Klobušický; *et al.*, 1995; Martinez-Machin; *et al.*, 1997; Rodero *et al.*, 1997; Schmidt *et al.*, 1997; Dorko *et al*, 1999).

In our study we tried to show prevalence of *C.tropicalis* in the population of babies and adult patients in relation to diagnosis and the infectious material during 5-year period.

Materials and Methods

334 samples of clinical material taken from patients accepted to different departments of Faculty hospital LF UPJŠ in Košice were examined for the presence of non-albicans Candida species. We examined the following: gynecological material, mouth, skin, wounds and plastic material swabs (drain, cannula, catheter), rarely blood, urine, sputum, ear swabs, and rectum and conjunction sac swabs.

C.albicans strains were eliminated from the set of examined fungi by means of a positive GT (germ tube) test (Otcenášek, *et al.*, 1990). *C.tropicalis* was identified by cultivation on Sabouraud glucose agar (SGA) (fy Imuna, Slovakia) and CHROMagar Candida (CHaC) (fy Mast Diagnostica, France) at 37 °C for 48 hours (Odds and Bernaerts, 1994; Dorko, *et al.*, 1999). A filamentous test on rice agar with Tween 80 was used to investigate the production of mycelium, way of its branching, shape, size, and arrangement of blastospores (Otčenášek, *et al.*, 1990). The ability of fungi to assimilate and ferment saccharides was investigated using auxanograms and zymograms (Otčenášek, *et al.*, 1990, Dorko, *et al.*, 1997). Microscopical examination of permanent Gram-stained mounts was used in preliminary evaluations.

Results

The microscopical Gram-stained mounts showed *C.tropicalis* cells as oval or spherical cells. However, microscopical appearance of cells is not an unambiguous indicator of possible indication of possible identification of this Candida because the shape of other fungi species is very similar. Branched hyphae, overgrown on both sides by droplet-shaped blastospores were observed on rice agar with Tween 80.

Table 1: Fermentation and assimilation of saccharides by *C.tropicalis*

Fermentation		Assimilation	
Glucose	+	Glucose	+
Galactose	+	Galactose	+
Saccharose	+	Saccharose	+
Maltose	+	Maltose	+
Lactose	-	Lactose	-
Raffinose	-	Raffinose	-
		Trehalose	+
		Mellibiose	-
		Cellobiose	v
		Xylose	+

Result: + positive, - negative, v variable

Table 2: Incidence of *C.tropicalis* in clinical materials

Material	No. of strains isolated from		Total No
	Children	Adults	
Gynecology material	1	19	20
Oral cavity	7	8	15
Cannula, catheter,drain	3	3	6
Wound	1	4	5
Skin	3	0	3
Blood	3	0	3
Urine	1	2	3
Sputum	1	1	2
Ear	1	0	1
Rectum	1	0	1
Conjunctival sac	0	1	1

C.tropicalis fermented glucose, galactose, saccharose and maltose and failed to ferment lactose and rafinose. Auxanograms of strains showed assimilation of glucose, galactose, saccharose, maltose, trehalose, xylose, and cellobiose (some strains exhibited slight or null assimilation of cellobiose) (Table 1). With regard to considerable differences in morphology of colonies it was impossible, on the basis of macroscopical examination, to determine unambiguously whether the species examined is really *C.tropicalis*. The fungi colonies growing on the SGA agar were

Table 3: Incidence of *C.tropicalis* in clinical materials from adults in relation to diagnosis

Diagnosis	Meterial	No.
Colpitils	vagina	5
	cervix	3
Abortus	vagina	1
	cervix	2
Gravidity	vagina	3
Endometritis	vagina	3
Fluor	cervix	1
Post partem	lichia	1
Tonsilitis	tonsils	1
Febrilitas	nasopharynx	1
Cerebral disease	nasopharynx	1
Hypertension	nasopharynx	1
Intoxication	tongue	1
Salpingitis	tongue	1
Kidney failure	tongue	1
Embolism		
Thrombosis	tongue	1
Osteotomy	wound	1
Pancratitis	wound	1
Umbilical hernia	wound	1
Osteoplasty	wound	1
Cholecystitis	drain	1
Surgical treatment	cannula	2
Uropathy	urine	1
Urinary tract		
Anomaly	urine	1
Conjunctivitis	conjunctiva	1
Bronchopneumonia	sputum	1

Table 4: Incidence of *C. tropicalis* in clinical material from pediatric population in relation to diagnosis

Diagnosis	Material	No.
Prematurity	nasopharynx	3
	skin	1
	blood	1
	cannula	1
	rectum	1
Coma	nasopharynx	1
	skin	1
	cannula	1
Bronchitis nasopharynx		2
pneumonia	sputum	1
	skin	1
Dialysis	catheter	1
	wound	1
M. Hodgkini	nasopharynx	1
Sepsis	blood	1
Malignity blood		1
Colpitis	vagina	1
Congenital urinary	urine	1
Tract disorders		
Otitis	ear	1

white, cream-coloured to gray and their surface was smooth, dull, or wrinkly. The centre of colonies was either convex or they exhibited slight to deep crater depressions surrounded with circular elevated border. The edge of colonies was most frequently circular and non-fibrous, or with short fibres, less frequently toothed, lobulated, fibrous or non-fibrous.

To facilitate the species identification, we used CHROMagar Candida which enables to distinguish individual fungi species even in mixed cultures on the basis of pigmentation of their colonies which is produced by reaction of species-specific enzyme with chromogenic substrate of the medium. *C.tropicalis* strains formed dark blue colonies on this cultivation media and purple to brown opacity zone was observed on agar in the colony surroundings. During the longer than 5-year period (from June 1993 to October 1998) we detected 334 species of non-albicans Candida. Of this number of fungi microorganisms *C.tropicalis* was proved in 60 samples (18%): gynecological material (n = 20), oral cavity swabs (n = 15), swabs from skin, wounds (n = 3,5, resp.), plasic materials (n = 6), blood (n = 3), urine (n = 3), sputum (n = 2) and sporadically swabs from ear, rectum and conjunctiva sac (one sample of each) (Table 2).

C.tropicalis strains were isolated from adults in 38 cases (63.3%) and from children in 22 cases (36.7%).

In the population of adults *C.tropicalis* was most frequently proved in gynecological material (lochia n = 1, cervix n-6, vagina n = 12), in diagnoses colpitis (n = 8), abortus (n = 3), gravidity (n = 3), endometritis (n = 3) fluor (n = 1) and in postparturient lochia (n = 1). *C.tropicalis* incidence in colpitis was proved 5-times from vagina and 3-times from cervix; at abortions (n = 3) twice from cervix and once from vagina. In fluor there was one positive swab from the cervix; in gravidity and endometritis three swabs from vagina (Table 3)

Examinations of the oral cavity showed *C.tropicalis* in 8 samples namely at tonsilitis (one strain from tonsils), recurrent fever vascular disease of the brain, arterial hypertension (one strain from nasopharynx for each), intoxication with medicines salpingitis, chronic failure of kidneys and arterial embolism and thrombosis (one strain for each of the diagnosis from a tongue swab) (Table 3). Four strains were isolated from post-surgical wounds, at osteotomy, pancreatis, umbilical hernia, and vacuole osteoplastics; one strain of drain at biliary tract disorders; two from post-surgical cannula, no diagnosis made; two strains from urine at obstructive and reflux uropathy and congenital urinary tract anomaly; one strain of each the conjunctiva sac at conjunctivitis and from sputum at bronchopneumonia (Table 3).

The highest incidence of *C.tropicalis*, i.e. 7 cases, was proved in children population in nasopharyngeal swabs at diagnosis prematurity (n = 3), bronchitis (n = 2), morbus Hodgkini (n = 1) and coma. Skin swabs showed presence of this fungi in skin swabs from premature baby, unconscious child and chid with pneumonia (n = 1 for each). Positivity of haemoculture for *C.tropicalis* was detected 3-times, namely at septic state, at diagnosis prematurity and malignant tumour (Table 4).

One strain of *C.tropicalis* for each of the following diagnoses was isolated from various examined materials: unconsciousness - swab from cannula, colpitis - vaginal swab, pneumonia - sputum prematurity - one swab from both endotracheal cannula and rectum, congenital urinary system disease - urine, otitis - ear swab; in addition to that two swabs from catheter and wound were positive at dialysis. The results obtained indicate that *C.tropicalis* was detected in 7 samples taken from premature children (Table 4).

The comparison of results obtained in the two examined groups showed that in the case of adult patiens *C.tropicalis* was most frequently detected at colpitis, altogether in 8 cases and of the materials examined highest positivity was in vaginal swabs (12 samples). The prevalence of *C.tropicalis* in the children population was the highest in premature babies (n = 7) and the same prevalence was observed also for nasopharynx samples. The prevalence of *C.tropicalis* in the oral cavity in adults reached 21% in comparison with 31.8% for children; the gynecological material was positive in 50% in adults while only one sample was positive in children, i.e. 4.5%. With regard to relatively low number of other diagnosis comparison of results between both groups showed no statistical significance.

Discussion

The present study and our previous investigations proved that *C.tropicalis* occupies the second place, right after *C.parapsilosis*, with regard to frequency of isolation, of all non-albicans Candida species (Dorko *et al.*, 1997; Dorko *et al.*, 1997). *C.tropicalis* was detected in 18% of samples taken from various clinical materals. This is by 3% higher result than that obtained by Al-Hedaithy and Fotedar (1997).

Our set of data showed the highest incidence of *C.tropicalis* in women patients with gynecological diagnoses (colpitis, abortus, graviditas, endometritis, fluor, postpartum). *C.tropicalis* was detected in 9 samples from patients with colpitis (Tab. 3, 4) which amounts to 2.7% of 334 non-albicans Candida species. This corresponds approximately to the percentage reported in the studies by Kaye and Kiraz (1994) and Klobušický *et al.* (1995) who identified this fungi in 2.2 - 2.4 % of cases . *C.tropicalis* and *C.krusei* are prevalent in the vulvo-vaginal area and the predisposition factors of vulvovaginal candidiasis are gravidity, diabetes mellitus, immunosuppression and others (Schmidt, *et al.*, 1997; Dorko *et al.*, 1999).

C.tropicalis is frequently detected in premature children admitted to neonatal intensive care units as indicated also in our study by seven positive findings (Table 4) (Kossoff *et al.*, 1998; Narang, *et al.*, 1998; Dorko, *et al.*, 1999). *C.tropicalis* is responsible for 16% candidiasis in children (Stamos and Rowley, 1995). It was detected 3-times in their blood, i.e. in 13.6% . Šimaljaková and Škutilová (1995) investigated mycotic infection in childhood and isolated *C.tropicalis* from the oral cavity in three cases - at tonsilitis, bronchitis and digestive tract disease - and once from skin at dermatitis . We also isolated *C.tropicalis* from the mouth cavity and skin of premature babies, unconscious patients, at bronchitis, pneumonia and oncological diseases (Table 4). Oral candidosis in newborns is mostly short-term easily treatable condition while in older children it occurs as a result of various predisposition factors, such as immunodeficiency, chemotherapy and similar and tendency to generalization of this problem was observed (Šimaljaková and Škutilová, 1995). Kockovská (1995) found 27 strains of *C.tropicalis* in sputa of adults suffering from pneumonia. This is significantly higher than the incidence found in our study (Table 3).

The specimens from patients with neoplasms were positive for *C.tropicalis* in two children only (Table 4). This was similar to the findings of Kunová *et al.* (1995) and Krcméry *et al.* (1998) but differed from the results of Wingard (1995), Abi-Said *et al.* (1997), Nucci *et al.* (1998) who showed as high as 18-48.5% prevalence.

Fungal infections of the urinary tract are frequent in hospitalized permanently catheterized patients. Fungal microorganisms are able to multiply in the urinary tract and form cell clusters (so-called bezoars) causing an obstruction in the ureter. This property has been ascribed not only to the principal fungal pathogen, C.albicans, but also to *C.tropicalis* (Scerpella and Alhalel, 1994, Guanter *et al.*, 1998). Al-Hedaithy and Fotedar (1997) and Oravcová *et al.* (1996) described 36 and 10% incidence of *C.tropicalis* in urine . However, our set of data showed the presence of this fungi only in 3 cases, i.e. in 5% of patients with obstructive reflux uropathy and congenital urinary system anomaly (n = 2) (Table 3,4).

Low incidence of fungi described in general for eye infections (Weissgold and Amico, 1996). A case of endophthalmitis, as a secondary complication in a patient with Candida endocarditis fitted with a pacemaker (Shmuely *et al.*, 1997). We were able to prove one strain of *C.tropicalis* in conjunctiva sac in a patient with conjunctivitis (Table 3).

C.tropicalis is capable of adhering to PVC materials and silicone protheses and exhibits also tigmotropism (ability to spread over the surface). These properties support the development of fungaemia (Inoue, *et al.*, 1995; Leow, *et al.*, 1997; Nikawa, *et al.*, 1997). Surface integrins are assumed to be responsible for the adherent properties (Bendel *et al.*, 1995). We were able to cultivate *C.tropicalis* in six cases from drain, catheter and cannula swabs (Table 3, 4).

High recovery of *C.tropicalis* reaching 8% was observed from oral cavity specimens of HIV-positive patients, patients with dentures, but also from those of healthy patients, as a part of physiological oral flora (Stenderup, 1990; Martinez-Machin, *et al.*, 1997; Dorko and Durovicová, 1998; Dorko, *et al.*, 1999). Due to increasing number of cardiosurgical interventions associated with transplantation of valves, *C.tropicalis* is a frequent etiological agent of endocarditis (Gerritsen, *et al.*, 1998; Roel, *et al.*, 1998). Incidence of *C.tropicalis* in the external environment in polluted water was described by Sláviková and Vadkertiová (1997). Contaminated milk has been identified as one of possible sources (Aalbaek *et al.*, 1994). It is important that *C.tropicalis* can be transmitted from one patient to another, however, transmission by hands of healthy medical personnel ,such is the case with other fungi species (*C.albicans, C.parapsilosis, C.krusei*) has not been proved (Pfaller *et al.*, 1998).

Examination of adult population proved *C.tropicalis* in 63.3% of specimens, most frequently in gynecological material in colpitis, and in children population in 36.7% of cases, most frequently in the oral cavity of premature babies. Identification of this fungus in the mycological laboratory is undemanding based on new differential cultivation media, such as CHROMagar Candida.

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