YEASTS OF CANDIDA GENUS IN DENTAL PLAQUE OF ECC-AFFECTED CHILDREN

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Background: The yeasts of the *Candida* genus represent an important part of the normal oral microflora, including dental plaque. Their ability to produce organic acids during the fermentation of carbohydrates participates in the cariogenic effect of dental plaque. **Aim:** The study aims to evaluate the occurrence of yeasts, especially *C. dubliniensis* in dental plaque samples obtained from children affected by Early Childhood Caries (ECC).

Design: In this study, 240 samples from patients suffering from ECC and 255 samples from healthy children were examined. Phenotypic methods (chlamydospore formation, characteristic growth on CHROMAgar Candida and Staib Agar, growth at 45 °C and latex agglutination) and the genotypic method (PCR with the species-specific primer pair targeted to *act1* intron and universal primer pair) were used for the differentiation between *C. dubliniensis* and *C. albicans*. **Results:** We isolated 167 yeast strains from patients with ECC (positivity 69.6 %) and only 97 yeast strains from control samples (38.0 %). The differences in yeast colonization of teeth between the two groups are significant ($p<0.01$). Of the total amount of 167 yeast strains isolated from ECC samples we identified 31 strains (18.5 %) of *C. dubliniensis*. The situation in the control group was different;only 2 *C. dubliniensis* strains (2.1 %) were identified. **Conclusion:** The results show that teeth of children with ECC are colonized with yeasts more frequently than teeth of healthy children. C. dubliniensis is a frequent part of dental plaque in ECC-affected children in contrast with healthy children.

Material and methods

Used strains:

The dental plaque samples (N=495) were obtained from children affected by Early Childhood Caries (ECC, N=240) and from caries-free children (N=255). The samples were cultivated on Sabouraud Agar (both Hi-Media, Mumbai, India) at 30 °C for 24 – 72 hours. The identification of isolated strains was performed using typical growth on CHROMAgar (CHROMAgar, France), by conventional morphological analysis, especially micromorphology on rice agar, and by commercially available biochemical kits CANDIDATest21 (Pliva-Lachema) and/or ID 32C system (bioMérieux, Marcy-l'Etoile, France). As controls the strains *C. albicans* CCM 8261, CCM 8320 (Czech Collection of Microorganisms) and *C. dubliniensis* CCY29-177-1 (Culture Collection of Yeasts) were used.

Differentiation between C. dubliniensis and C. albicans:

CHROMAgar (CHROMAgar, France): cultivation at 30 °C for 48 hours; *C. albicans* forms light-green colonies, *C. dubliniensis* forms dark-green colonies.

Growth at 45 °C: *C. dubliniensis*, contrary to *C. albicans*, fails to grow on Sabouraud agar at 45 °C.

CHROMAgar

C. albicans

C. dubliniensis

Staib agar: The strains were inoculated on Staib agar (containing pulverized *Guizotia abyssinica* seed) and incubated at 30 °C for 72 h. *C. dubliniensis* strains form rough colonies with abundant hyphae and chlamydospores on Staib agar; *C. albicans* isolates form smooth colonies without hyphae and chlamydospores.

Staib agar

C. albicans

C. dubliniensis

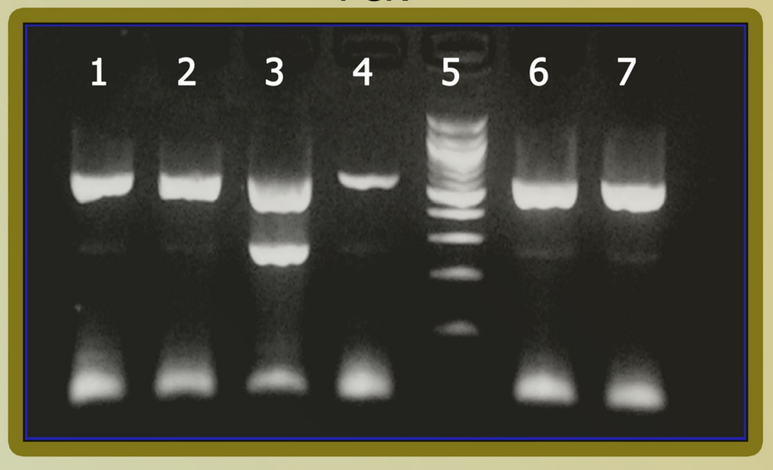
Latex agglutination test to identify *C. dubliniensis*: BICHRO-Dubli (Fumoze Diagnostics)

PCR: The 20 µL Phusion™ Flash High-Fidelity PCR Master Mix (Finnzymes, Francie)

species-specific primers targeted at *C. dubliniensis act1* intron:
Uni-f (5´-GCATATCAATAAGCGGAGGAAAAG-3´),
Uni-r (5´-GGTCCGTGTTTCAAGACG-3´) – product 614 p.b.
Internal control : Act-f primer (5´-GTATTTGTCGTTCCCCTTTC-3´),
Act-r primer (5´-GTGTTGTGTGCACTAACGTC-3´) - product 216 p.b.

PCR reaction was performed in a termal cycler PTC-200 (BioRad).
Kruskal – Wallis and McNemar´s tests were used for statistical evaluation (Statistica for Windows v. 8.0).

PCR



C. albicans - 1,2,4,6,7 *C. dubliniensis* - 3 Weight marker - 5

Results and conclusions

We isolated **264** yeast of *Candida* genus in dental plaque samples from children affected by ECC (n=167) and from caries-free children (n=97). The differences in yeast colonization of teeth between the two groups are significant ($p<0.01$).

Table 1. Yeasts of *Candida genus* in the dental plaque of children

	Samples	*Candida* sp.	*C. dubliniensis*
ECC	240	167 (69.6%)	31 (18.5%)
healthy	255	97 (38%)	2 (2.1%)

Statistical significant difference ($p<0.01$) *in yeasts colonization.*

197 isolates grew as green colonies on chromogennic medium in CHROMAgar Candida and formed pseudomycelium and chlamydospores on rice agar. These strains were considered *C. albicans*/*dubliniensis*.

Phenotypic methods (characteristic growth on CHROMAgar Candida and Staib Agar, growth at 45 °C and latex agglutination) were used for the differentiation between *C. dubliniensis* and *C. albicans*. Identification of *C. dubliniensis* was confirmed by means of genotypic latex agglutination and by PCR targeted at *C. dubliniensis act1* intron.

We identified 33 strains (12.5 %) of *C. dubliniensis* from the total amount of isolated strains. Two *C. dubliniensis* strains (2.1%) were from 97 from caries-free children isolates, 31 *C. dubliniensis* strains (18.5 %) were from 167 isolates from children affected by ECC. The differences in incidence of these yeast species are significant ($p<0.01$).

The results show that teeth of children with ECC are colonized with yeasts more frequently than teeth of healthy children. *C. dubliniensis* is a frequent part of dental plaque in ECC-affected children in contrast with healthy children.

Table. 2 Phenotypic methods used for differentiation between *C. dubliniensis* and *C. albicans*

n = 197		CHROMAgar			Growth at 45°C			Staib agar		
		0	+/-	+	+	+/-	0	0	+/-	+
PCR/latex agglutination	0	122	28	14	141	17	6	146	19	2
	+	3	6	24	0	4	29	0	2	31

Reliability of simple phenotypic methods used for differentiation between *C. dubliniensis* and *C. albicans* is limited. Therefore, these methods are suitable only for screening of clinical strains. The best way to perform the phenotypic identification of *C. dubliniensis* is to combine phenotypic methods as they are not sufficient when applied independently.

PCR and latex agglutination are more reliable in comparison with above mentioned method and they are suitable for confirmation of the results.

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