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# *Differentiation by RAPD-PCR of Candida albicans isolated from upper respiratory tract in elderly people from care center*

Różnicowanie przy użyciu RAPD-PCR szczepów *Candida albicans* izolowanych z górnych dróg oddechowych osób w wieku podeszłym przebywających w zakładzie opiekuńczym

## INTRODUCTION

Elderly people, aged 65 years old or older, are predisposed to fungal infections caused especially by yeast species belonging to *Candida* spp., mainly *Candida albicans*. Among them the most common are respiratory tract candidiasis. Such infections are usually endogenous in origin due to colonization of nasopharyngeal mucosa by *Candida* spp. Increased susceptibility of elderly people to colonization of mucous membrane of upper respiratory tract by *Candida* spp. may be related to various factors, including age-dependent immunosupression, atrophic changes in mucous membrane, favouring adhesion of microorganisms, slight general state of organism due several chronic diseases, insufficient hygiene of oral cavity and hands, bad diet, frequent cases of hospitalization, frequent intake of antibiotics with a broad spectrum activity [2, 4, 6, 8].

Recent advances in molecular techniques have generated several typing methods based on PCR for genetic assessment of genetic relatedness of bacterial or fungal strains. One of them, RAPD-PCR analysis is a reliatively easy, reproducible and reliable technique that can be useful in providing genetic fingerprints for the differentiation of *C. albicans* [3, 7, 9].

The aim of this study was to estimate the genetic diversity by RAPD-PCR of *C. albicans* isolated from the upper respiratory tract in elderly people from a care center in order to assess spreading of these strains among people staying in close population.

## MATERIAL AND METHODS

A total of 75 isolates of *C. albicans* colonizing the upper respiratory tract in 51 elderly people (aged 65–97 years) from a care center were included in these studies. The DNA from the isolates was prepared using GeneMATRIX Yeast DNA Purification Kit ($EUR_X$) according to the manufacturer's procedure. RAPD-PCR method was performed with RSD12 primer (5'-GGTCCGTGTTTCAAGACG-3') [7, 10]. PCR reactions were carried out in the thermocycler and amplification conditions were: 40 cycles

of denaturation at 94ºC for 30 s, followed by primer annealing at 57ºC for 2 min and elongation at 72ºC for 2 min. Final extended elongation at 72ºC lasted 15 min. A polymerase chain reaction was performed in 0.5 ml microcentrifuge tubes in a final reaction mixture containing 100–400 ng of *C. albicans* DNA as template, 10 x PCR buffer for Taq DNA polymerase (Fermentas), 1 u/µl of Taq DNA polymerase (Fermentas), 200 µM dNTPs (Fermentas), 2.5 mM $MgCl_2$ (Fermentas), 1.25 µM of primer RSD 12 (Proligo primer&probes).

For each experiment, the sizes of DNA fragments amplified by PCR were determined by direct comparison with the DNA marker – 100 bp Ladder Plus (Fermentas). Control tubes without template DNA were included in each run and reproducibility was checked for each reaction. The PCR products were electrophoresed in agarose gels (1.5%) at 120 V for approximately 100 min at room temperature in TBE buffer (Tris Borate Electrophoretic Buffer, 89 mM Tris/HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.0) (Sigma). Reaction products were detected by ethidium bromide and visualized with UV light.

The different banding positions of RAPD fingerprinting patterns of isolates of *C. albicans* were analysed using the BioGene (Polygen) program. For the analysis of relationships among the strains, BioGene constructs dendrograms by the unweighted pair group method. Each lane pattern was compared to every other pattern through computations of a similarity coefficient, which compares the band positions.

## RESULTS

In the current study, the value of a similarity coefficient ≥ 80% was arbitrarily used as the threshold for clustering of similar genotypes, since it is roughly halfway between the mean value for dissimilarity and identity [7]. On the basis of RAPD-PCR profiles, among 75 strains of *C. albicans*, 46 genotypes were defined within the overall yeast population, including 11 clusters containing from 2 to 9 isolates, which comprised 40 (53.33%) isolates and 35 (46.67%) genotypic unique strains (Figure 1).

## DISCUSSION

RAPD-PCR method is a useful tool for confirming genetic and filogenetic diversity among *C. albicans* and may be applied as a valuable method in the epidemiological purposes [2, 4, 5]. This technique is easy to perform, simple, versatile and a useful discriminatory method for epidemiological studies of *Candida* spp. [3, 4, 6, 8, 9, 10].

In the present study, we observed that RAPD-PCR technique pointed out the genomic variability within the *C. albicans* isolated from upper respiratory tract of elderly people. Our data obtained with primer RSD12 indicate significant differences in RAPD-PCR profiles between *C. albicans* strains isolated from the upper respiratory tract in elderly people from a care center, despite staying in close population. The obtained data suggest that with even genotypes of the yeast isolates from the same person differed significantly, suggesting continuous exchange of *C. albicans* and deficiency of predominant strains.

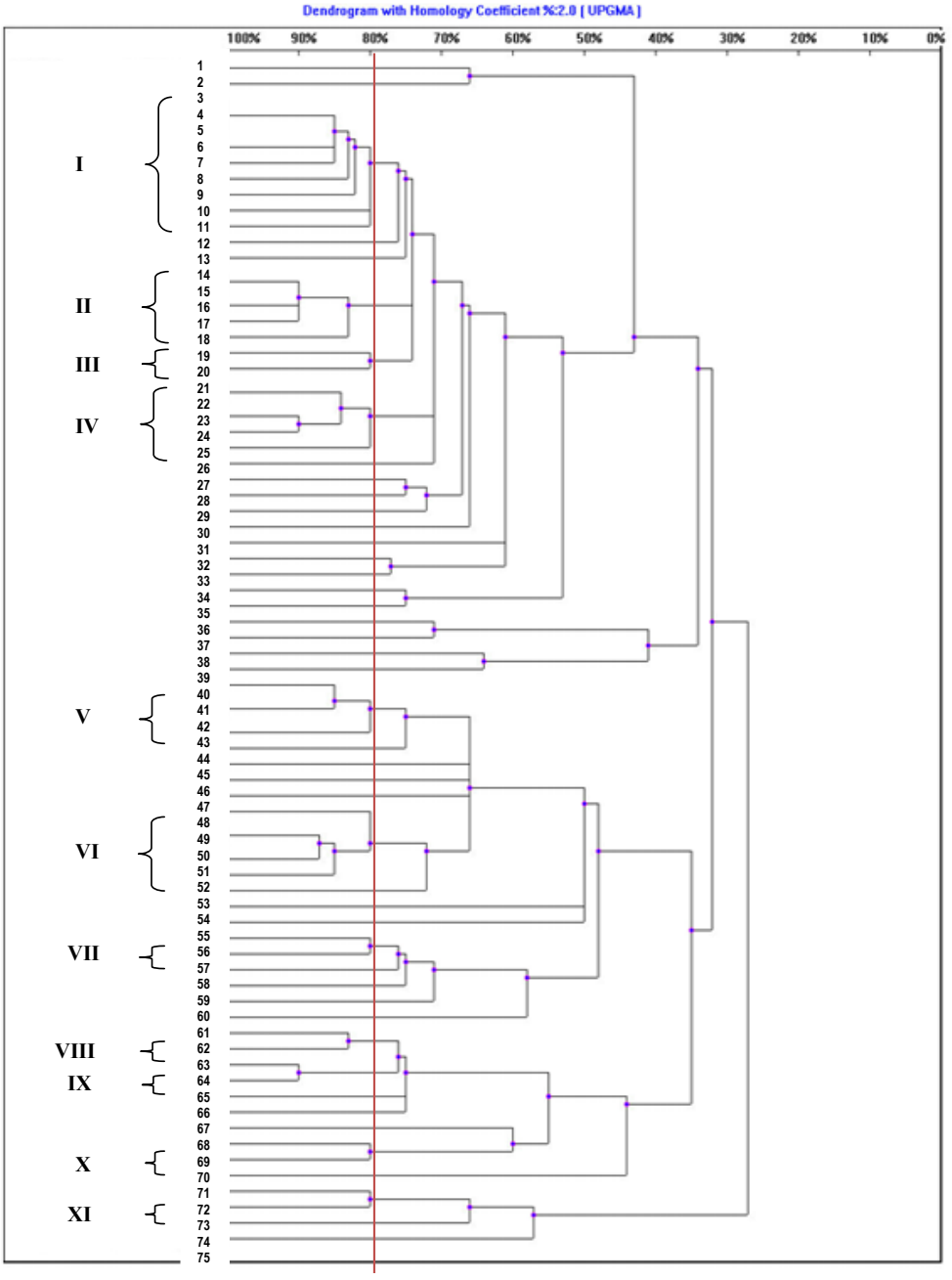


Fig. 1. Dendrogram based on RAPD-PCR data for C. albicans isolates from upper respiratory tract of elderly people staying at care center. Clusters were described as I-XI

Samaranayake et al. [7], using RAPD-PCR technique with two different primers RSD10 and RSD12, were able to determine the clonal variability of 443 colonizing oropharyngeal *C. albicans* strains obtained from 16 HIV-infected individuals. *C. albicans* isolates formed clusters comprising 2 to 3 or more strains at values of a similarity coefficient ≥ 80%. In turn, Waltimo et al. [10], using primer RSD6 and also the same RSD12 to assess genetic diversity of *C. albicans* isolates from root canal infections, found 31 genotypes among the 37 isolates.

Our data and those from literature suggest that genetic diversity is typical of *C. albicans* isolates, irrespective of their origin.

## CONCLUSIONS

The analysis of *C. albicans* genotypes by RAPD-PCR method revealed a large genetic variability among the strains isolated from the upper respiratory tract of elderly people staying at a care center.

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SUMMARY

Elderly people, aged 65 years old or older, are predisposed to fungal infections caused especially by *Candida* spp., mainly *Candida albicans*. Among them the most common are respiratory tract candidiasis. The aim of this study was to estimate the genetic diversity by RAPD-PCR of *C. albicans* isolated from the upper respiratory tract in elderly people from a care center in order to assess spreading of these strains among people from close population. Among 75 strains, 46 genotypes were defined. 11 clusters comprising 40 (53.33%) isolates at the value of similarity coefficient ≥ 80% were formed. The remaining 35 (46.67%) isolates represented individual genotypes.

Key words: differentiation, RAPD-PCR, *Candida albicans,* upper respiratory tract

STRESZCZENIE

Osoby w wieku podeszłym, powyżej 65 roku życia, są szczególnie predysponowane do infekcji grzybiczych wywoływanych przez *Candida spp.*, głównie *Candida albicans*. Należą do nich najczęściej kandydozy dróg oddechowych. Celem badań była ocena genetycznego zróżnicowania szczepów *C. albicans* izolowanych z górnych dróg oddechowych osób starszych przebywających w domu opieki społecznej w celu oceny rozprzestrzeniania się tych szczepów wśród osób przebywających w zamkniętej populacji. Uzyskane wyniki wskazują na znaczne zróżnicowanie genetyczne izolatów *C. albicans*. Wśród 75 szczepów *C. albicans* stwierdzono 46 genotypów. Wyróżniono 11 typów genetycznych obejmujących 40 (53,33%) izolatów o współczynniku pokrewieństwa ≥ 80% oraz 35 (46,67%) szczepów unikatowych.

Słowa kluczowe: różnicowanie, RAPD-PCR, *Candida albicans,* górne drogi oddechowe