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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Study of skin and nail *Candida* species as a normal flora based on age groups in healthy persons in Tehran-Iran

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Summary The skin is the body's largest organ that hosts heterogeneous inhabitants. Until now, the diversity of the cutaneous microbiome was mainly investigated for bacteria and there is a little information about the skin fungal flora. Also, among skin fungal flora, *Candida* is found as a main member whose distribution is affected by sex, age, climate. In this study, differences in *Candida* community structure associated with 9 different skin sites of 238 healthy people during 10 months from July to March 2016, are described. These subjects were divided by age into 4 groups: infants, children, adults and geriatrics. The collected samples were examined by culture on Sabouraud Chloramphenicol Agar and CHROM-agar *Candida*. For precise identification of species ITS1-5. 8S-ITS2 rDNA regions were sequenced where needed. The frequency of *Candida* species was significantly different between age groups. The most *Candida* isolations were related to the elderly age group and the fewest in the infants. *C. parapsilosis* virtually, was the predominant isolated species in all age groups. This study showed no statistically significant effect of the subject's sex on *Candida* population resident on human skin surface.
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Introduction

Skin as the largest organ of human body is significantly colonized by a variety of bacterial and fungal population [1,2]. Today the percent of opportunistic fungal infections is increasing, so when considering the role of skin as a reservoir from which infection of a susceptible host can occur, determining the fungal microbiome specially *Candida* is important [3]. Two of the most factors influencing the complex of inhabitants on the skin are gender [4] and age [5,6]. There are more data concerning bacterial cutaneous flora than fungal flora, and among fungal flora, *Candida* species may be one of the important opportunistic agents residing on the skin with the potential to create different kinds of candidiasis [7,8].

There is little information about *Candida* distribution inhabiting on human skin in the word, and our information is related to more than half a century ago [7].

It should be noted, this is the first study about this subject in Iran.

The purpose of this study were to create new information about the type and percentage of *Candida* population in each site of the human skin, attempting to determine what influences the individual's age and sex have on the composition and percentage of cutaneous *Candida* community in Iranian people. As we know, Iran is a tropical country and fungal organisms can significantly growth over this condition.

Materials and methods

Ethics statement

This study was approved by ethical committee of Tehran University of medical science (the number of Ethics Committee protocol: IR.TUMS.SPH.REC.1395.1339). A written informed consent was obtained from all subjects or their guardians prior to sample collection. All data were de-identified.

Sampling

A total of 238 healthy people, in four age groups, were studied. These subjects included 119 males and 119 females, with equal gender distribution in all age groups. All samples were selected from 5 areas of Tehran (North, South, East, West and Center of these city). The distribution of subjects in each group was as follows:

- 55 healthy, full term babies between the ages of 4 to 15 days, including infants referring to the health house;
- 60 children aged between 1 and 12 years old. Most were drawn from schools and kindergartens;
- 62 healthy adults, aged between 18 and 45 years old. This group was composed of students of Tehran University, factory workers, health workers and housewives;
- 61 old people over 60 years age which often consist of retirements.

At the time of sampling, the age and gender of each subject were recorded. The subjects have not washed their hands, feet, or other areas just before sampling and the sampling was made after the activities of the day. The following areas were sampled by means of a cotton-tipped swab moisten with sterile serum physiology: the forehead, dorsum of hands, dorsum of feet, finger nails, toenails, the axilla, the groin, the interdigital spaces of hand and foot and the submammary space in women.

All swabs were cultured on Sabouraud Chloramphenicol Agar (SC, Merck, Germany). All cultures were incubated in 25 °C for 4 days. Isolated colonies were identified by growth on Corn Meal Agar Tween 80 (Micro media, Hungary) and CHROM-agar Candida (Paris, France) in 30 °C after 4 days. Chlamydospore formation by *C. albicans* on cornmeal agar can be a differential factor for this species.

In this study, for a correct determination of samples which were not detectable by mycological techniques, DNA sequencing was performed.

Molecular technique

DNA extraction

An aliquot of 100 μL of cell suspension was transferred to microtubes and incubated at 100 °C in a boiling water-bath for 10 min, then centrifuged at 5000 × g for 5 minutes. The upper aqueous layer (containing the DNA) was carefully transferred to a clean tube and was used for PCR.

PCR conditions and sequencing

PCR amplification of ITS1-5. 8S-ITS2 rDNA regions was performed [9]. Positive PCR products were sent for sequencing at Bioneer Advanced Nucleic Acids core facility. The ITS sequences were then parsed from the contiguity and separately used to perform individual nucleotide–nucleotide searches using the BLASTn algorithm at the NCBI website (http://www/ncbi.nlm.nih.gov/BLAST/). Fungal identifications were made based on maximum identities $\geq$ 99% and query coverage $\geq$ 98% with this method.

Statistical tests

In this study, one tail Chi-square test was performed for each analysis.

Results

In this study, on each medium that the growth of *Candida* was positive, one isolate was identified. However, from a few mediums related to some sites of the skin, two isolates were detected. Also for determination of 40 samples which were not detectable by culture, DNA sequencing was performed.

Among the studied population, divided into 4 groups by age including infants, children, adults and the elderly, 162 persons were positive for *Candida* isolation. The highest prevalence of *Candida* isolation was related to the elderly age group ($n = 83$, 51.2%) and the lowest prevalence was related to the infants ($n = 11$, 6.7%) and showed the age of subjects was significantly effective on cutaneous *Candida* community ($P < 0.001$). Also, in this study, *C. parapsilosis* ($n = 61$, 37.6%) among all species was predominant in virtually all age groups follow by *C. krusei* ($n = 37$, 22.8%), *C. glabrata* ($n = 33$, 20.3%), *C. albicans* ($n = 26$, 16%) and finally *C. tropicalis* ($n = 5$, 3%).

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