

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

Abstract

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 and *Trichosporon* is occasionally found as normal flora of skin. In this study, differences in *Trichosporon* 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 Sabouraud Cycloheximide Chloramphenicol Agar. For precise identification of isolates in the species level, ITS1-5.8S-ITS2 r DNA regions were sequenced. The frequency of *Trichosporon* species was not significantly different between age groups. The most *Trichosporon* isolations were related to the adult age group and the fewest in the infants. *T. asahii* was the predominant isolated species in all age groups. This study showed statistically significant effect of the subjects' sex on *Trichosporon* population resident on human skin surface.

Keywords: skin residents, cutaneous *Trichosporon* composition, different age groups, DNA-sequencing, culture, Microbial epidemiology, Iran

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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} and *Trichosporon* is a normal fungal inhabitant of the skin. Opportunistic infections by this fungus has been increasingly often report in immune compromised patients.³⁻⁶ Two of the most factors influencing the complex of inhabitants on the skin are gender⁷ and age.^{8,9} There is little information about *Trichosporon* distribution inhabiting on human skin in the world and 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 *Trichosporon* 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 *Trichosporon* 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 were including 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 this 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 house wives.
- 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 haven't 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 moistened 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 sub mammary 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 then were cultured on Sabouraud Dextrose Agar with chloramphenicol and cycloheximide (Micro media, Hungary) in 25°C after 4 days. This medium is a selective solid medium for differentiation *Trichosporon* spp from *Geothricum candidum*. Unlike *Geothricum candidum*, *Trichosporon* isolates grow on this medium. In this study for a correct determination of isolates in the species level, DNA sequencing was performed.

Molecular technique

DNA Extraction: An aliquot of 100 µL of cell suspension was

transferred to micro tubes and incubated at 100°C in a boiling water-bath for 10 min, then centrifuged at 5,000 x 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 were performed.¹⁵ Positive PCR products were sent for sequencing at Bioneer Advanced Nucleic Acids core facility. The ITS sequences were then parsed from the coating 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 ≥ 99% and query coverage ≥ 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 *Trichosporon*

was positive, one isolate was identified and for precise identification of isolates in the species level, DNA sequencing was performed. Among the studied population, divided into 4 groups by age including infants, children, adults and the elderly, 12 persons were positive for *Trichosporon* isolation. The highest prevalence of *Trichosporon* isolation was related to the adults age group (n=7, 58.33%) and the lowest prevalence was related to the infants (n=1, 8.33 %) and showed the age of subjects was not significantly effective on cutaneous *Trichosporon* community (p > 0.01). Also in this study

T. asahii (n=11, 91.66%) among all species was predominant in all age groups follow by *T.coremiiforme* (n=1, 9.09). It should be noted there was not a significant difference between isolated species according the age (Table 1). In this study 238 healthy people including 119 males and 119 females were examined. From 12 persons who were positive for *Trichosporon* isolation, the isolation of *Trichosporon* was significantly different between males (n=4, 33.33%) and females (n= 8, 66.66%) and there was significant difference in cutaneous *Trichosporon* population between the genders. (p = 0.01) (Table 2).

Table 1 The frequency of *Trichosporon* species in different age groups

Age group	Infants		Children		Adults		Elderly		Total	
	Number	%	Number	%	Number	%	Number	%	Number	%
<i>T. asahii</i>	0	0	1	100	7	100	3	100	11	91.66
<i>T.coremiiforme</i>	1	100	0	0	0	0	0	0	1	8.33
Total	1	100	1	100	7	100	3	100	12	100

Table 2 The frequency of *Trichosporon* species in regard to gender

Gender	Men		Women		Total	
	Number	%	Number	%	Number	%
<i>Trichosporon</i> species						
<i>T.asahii</i>	4	100	7	87.5	11	91.66
<i>T.coremiiforme</i>	0	0	1	12.5	1	8.33
Total	4	100	8	100	12	100

Considering *Trichosporon* isolation in regard to anatomic sites, from 19 positive sites for *Trichosporon* isolation, the highest prevalence of *Trichosporon* isolation was related to the axilla (n=4, 21.5%) and the groin (n=4, 21.5%) and the lowest prevalence was

related to the sub mammary space in women (n= 0, 0.%) and dorsum of hands (n= 0, 0.%). Also in this study, *T.asahii* (n= 15, 78.94%) showed the highest prevalence of *Trichosporon* isolations in all studied anatomical sites (Table 3).

Table 3 The frequency of *Trichosporon* species in regard to anatomic site of the body

<i>Trichosporon</i> species	<i>T.asahii</i>		<i>T.coremiiforme</i>		Total	
	Number	%	Number	%	Number	%
Forehead	1	6.66	1	25	2	10.52
Dorsum of hands	0	0	0	0	0	0
Dorsum of feet	1	6.66	0	0	1	5.26
Finger nail	3	20	0	0	3	15.78
Toe nail	3	20	0	0	3	15.78
Axilla	3	20	1	25	4	21.05
Groin	3	20	1	25	4	21.05
Inter digital space of feet	1	6.66	1	25	2	10.52
Sub mammary space	0	0	0	0	0	0
total	15	100	4	100	19	100

Discussion

In this study, we characterized the cutaneous *Trichosporon* composition and the percentage of *Trichosporon* species inhabiting on each site of human skin by focus on show the effect of the individual's age and sex on it. Our study showed that the lowest prevalence of *Trichosporon* isolation is related to the infant age group, this finding may be a result of this fact that the cases were at the age of 4-15 days and the chance of skin colonization with *Trichosporon* during this time is less than other age groups. Fetal skin is sterile, but *Trichosporon* colonization occurs through the hands of health care workers, parents and the infants contact with objects and equipment related to neonatal care.^{10,11} The highest prevalence of *Trichosporon* isolation recorded from the skin of adult individuals. The main reason for this is possibly due to high level of daily activities in this age group and their greater contact with source of pollution compared with another age groups.¹² In our study the frequency of *Trichosporon* isolation was significantly greater in females compared to males. Physiological and anatomical differences between male and female and cutaneous environments such as sweat, sebum and hormone production, partially account for the microbial differences seen between the genders.¹³ During this investigation the maximum amount of *Trichosporon* isolation in regard to anatomic site of the skin was related to the axilla and the groin. These regions are higher in temperature and humidity, which encourages the growth of these yeasts, the increased chance of yeasts isolation from this anatomical site is explainable.¹⁴ Isolation of *T.asahii* as the commonest species in all sites of the skin in our study was noteworthy. The prognosis of infection caused by this yeast is very poor, its mortality being approximately 70%. This is higher than that of candidacies, with a mortality of 40%.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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