

Original Research Article

A study on *malassezia* micro flora in the skin of healthy individuals in North Kerala, India

Remya V. S.¹, Arun B.^{2*}, Sheeba P. M.³, Pratyusha Kokkayil³

¹Department of Microbiology, Pariyaram Medical College Kannur, Kerala, India

²School of Health Sciences, Kannur university, kannur, Kerala, India

³Department of Microbiology, Government Medical College Palakkad, Kerala, India

Received: 14 August 2017

Accepted: 07 September 2017

*Correspondence:

Dr. Arun B.,

E-mail: arunat1976@gmail.com

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ABSTRACT

Background: *Malassezia* spp are accounted for approximately 60-90% of the total cutaneous fungal micro biota. An enhanced understanding of the distribution of *Malassezia* spp in healthy individuals is necessary to gain insight to involvement of these species in human skin disorders particularly in pityriasis versicolor. The aim of this study was to assess the *Malassezia* micro flora of healthy individuals and to identify species variation with different body sites, gender and age groups.

Methods: A total of 120 apparently healthy men and 120 women without any skin diseases or who were not treated for *Malassezia* associated skin diseases were selected for the study. The specimens consisted of scrapings collected from chest, back and neck from each control subject totaling 720 sites.

Results: The recovery rate of *Malassezia* was positive in case of 452 (62.78%) of 720 sites sampled, while the remaining 268 (37.22%) were found to be negative. Recovery rate of *Malassezia* was highest in chest with 83.33% positive cases, followed by the back with 73.33% and the neck 31.67%. *M. globosa* was identified as the common species (46.46%) followed by *M. furfur* (25%) and *M. restricta* (22.56%). No significant difference was present ($P > 0.05$) in distribution of species with different body sites, gender and age groups.

Conclusions: The overall colonization rate of *Malassezia* spp in normal healthy individuals found in this study was 62.78%. *M. globosa* was identified as the common species (46.46%).

Keywords: *Malassezia* species, Normal microflora

INTRODUCTION

The skin is an ecosystem colonized by a diverse milieu of micro-organisms.¹ They are primarily found in the stratum corneum and hair follicles in humans as well as animals. In human skin, *Malassezia* spp are accounted for approximately 60-90% of the total cutaneous fungal micro biota where they thrive on sebum rich in triglycerides and esters.² Currently, the genus *Malassezia* has 14 species. These include *M. furfur*, *M. sympodialis*, *M. obtusa*, *M. globosa*, *M. restricta*, *M. slooffiae*, *M.*

pachydermatis, *M. dermatis*, *M. japonica*, *M. yamatoensis*, *M. nana*, *M. caprae*, *M. equina*, and *M. cuniculi*.² In healthy human beings, under steady state conditions they remain in equilibrium with skin as commensals.³ But sometimes under predisposing conditions, they proliferate and grow in hyphal forms without inflicting inflammation as in pityriasis versicolor or are involved in the pathogenesis of diseases with noticeable inflammation. (seborrheic dermatitis, atopic dermatitis, and psoriasis etc.).^{3,4} They are also found to be involved as an associated agent or contributory factor in

several other clinical conditions such as onychomycosis, folliculitis, neonatal cephalic pustulosis, confluent and reticulate papillomatosis of gougerot–carteaud.⁵ Since the new descriptions, several recent studies have been carried out worldwide to study the distribution of the newly defined species of *Malassezia* on healthy adult human skin in which variable results have been reported among studies from different geographical regions.⁶

An enhanced understanding of the distribution of *Malassezia* spp in healthy individuals is necessary to gain insight to the involvement of these species in human skin disorders particularly in pityriasis versicolor. The aim of this study was to assess the *Malassezia* micro flora of healthy individuals and to identify species variation with different body sites, gender and age groups. This is the first study to analyze the distribution of *Malassezia* spp normal healthy skin of human beings from North Malabar area, Kerala, India.

METHODS

This study was conducted at a tertiary care hospital in North Kerala and School of health sciences, Kannur, Kerala. A total of 120 apparently healthy men and 120 women without any skin diseases or who were not treated for *Malassezia* associated skin diseases were selected for the study. They were grouped according to their age as (0–10), (11–20), (21–30), (31–40), (41–50), (> 51) where each group consisted of 40 subjects (20 men and 20 women). The specimens consisted of scrapings collected from chest, back and neck from each subject totaling 720 sites.

All samples were cultured on to modified Dixon’s agar (mDixons agar). The cultures were incubated at 32 °C for seven days. Once the growth occurred gram staining was performed and colonies formed were subjected to speciation by morphological, physiological and biochemical tests as described by the methodology of Guillot et al, Mayser et al.⁹

RESULTS

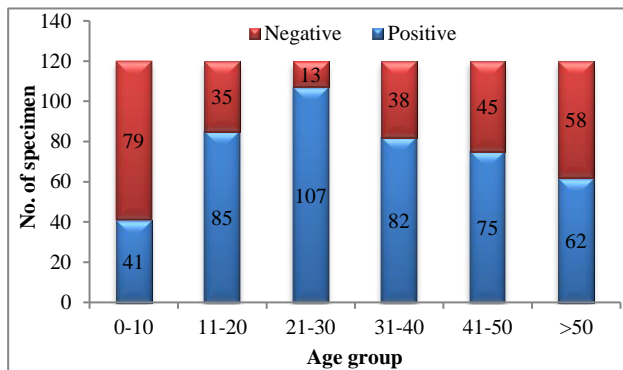


Figure 1: Isolation rate of malassezia spp according to age group.

The recovery rate of *Malassezia* was positive in case of 452 (62.78%) of 720 sites sampled, while the remaining 268 (37.22%) were found to be negative. The positive culture rate in normal healthy individuals was highest in the age group of 21– 30 with 107 isolates (89.2%) followed by 11– 20 with 85 isolates (70.8%) and in, 31– 40; 82 isolates (68.3%), as shown in the Figure 1.

Males showed higher culture rates than female counterparts in all age groups and this difference is not statistically significant. (p >0.5). According to different sites sampled, the positive rate of isolation of different *Malassezia* spp in culture was highest in chest with 200(83.33%) positive cases, followed by the back with 176 (73.33%) and the neck76 (31.67%). Higher culture positive rates of *Malassezia* spp were shown in male subjects than female counterparts in all body sites except back and this difference was not found to be statistically significant (P>0.05) as shown in the Figure 2.

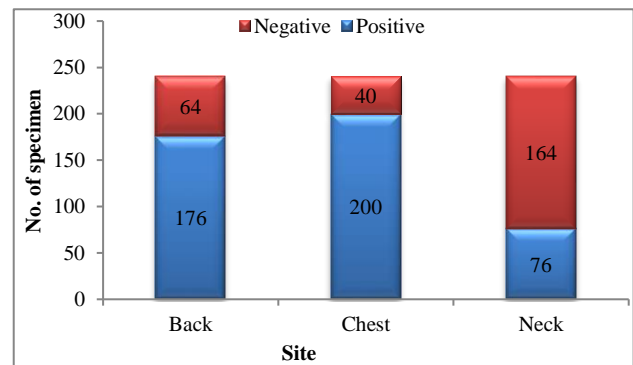


Figure 2: Isolation rate of malassezia spp according to body sites in healthy individuals.

In healthy individuals, among different *Malassezia* spp isolated in six different age groups, *M. globosa* was identified as the common species with 210 isolates (46.46%) followed by *M. furfur* at 113 (25%) and *M. restricta* 102 (22.56%). *M. sympodialis* with 17 isolates (3.76%), *M. obtusa* with 5(1.11%), *M. slooffiae* with three (0.66%) *M. pachydermatis* with two (0.44%) were less frequently isolated (Figure 3).

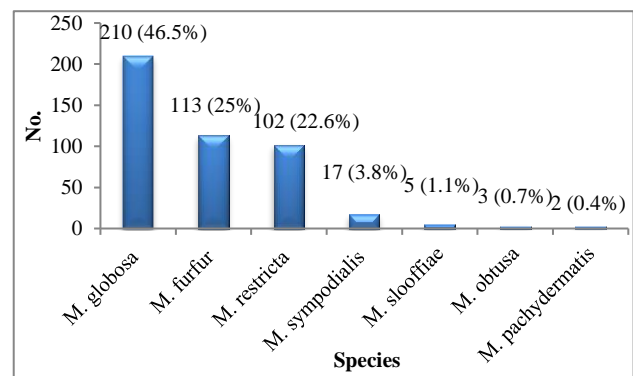


Figure 3: Isolation of different malassezia spp in healthy individuals

M. globosa was the predominant isolate from all age groups followed by *M. furfur* in 0–10, 21–30, 41–50, and >50 age groups. Conversely in age group of 11–20 and 31–40 isolation of *M. restricta* was higher than *M. furfur*. *M. pachydermatis* was isolated from 41–50 age group. The results from each body site (chest, back, neck) indicated that *M. globosa* as the most predominant species in all studied sites (46.46%). In neck and chest *M. globosa* was followed by *M. furfur*. *M. restricta* and other species were less frequently associated with neck. In back, *M. globosa* followed by *M. restricta* and *M. furfur* were involved. *M. globosa* was most commonly isolated from chest followed by back and neck. *M. furfur* was also isolated more commonly from chest followed by back and neck. On the other hand, *M. restricta* was isolated frequently from back followed by chest and neck. Of the 17-isolated species of *M. sympodialis* 13 of them were isolated from chest and the remaining four from neck. Other species were less frequently isolated.

DISCUSSION

Malassezia spp are known to be a part of cutaneous microbiota. They are isolated mainly from the sebaceous rich areas on the face, scalp, and upper trunk and the extent of colonization on the limbs was comparatively lower.^{10,11} Their absolute requirement for lipids and mesophilic nature (optimum temperature being 30–35°C) might explain their presence only on the skin of warm blooded animals.¹² The overall colonization rate of *Malassezia* spp in normal healthy individuals found in this study was 62.78%. This is in agreement with the studies done in normal healthy Korean subjects at 62.4% and 63.6%.^{13,14} An Iranian study showed similar rates at 62%.¹⁵ Higher rate of *Malassezia* colonization compared with the present study was also noticed in studies done on healthy human skin in Bosnia and Herzegovina (65%) and Sweden (84%).^{16,17} Studies done in healthy Iranian, Indian and Tunisian healthy normal subjects yielded lower rate of colonization ranging from 31.2%–43%.^{18–20}

This study revealed only pure cultures of a single *Malassezia* isolated from one site in healthy individuals. This is in variance with a study done on Tunisian healthy normal subjects in which (13.32%) showed mixed cultures of different *Malassezia* spp including *M. globosa* + *M. sympodialis* (4.44%), *M. globosa* + *M. furfur* (4.44%), *M. globosa* + *M. restricta* (3.33%) and *M. globosa* + *M. slooffiae* (1.11%).²⁰ Mixed cultures were also observed in a couple of studies from normal healthy Korean subjects based on molecular based studies.^{14,13} However, diverse sites yielded different species.

Similar to the other studies done in normal Korean healthy men and women, and when compared with other age groups, the population density of *Malassezia* was significantly higher in the age group of 21–30 years (89.2%).^{13,21} Marcon MJ and Powell DA in 1992 opined that their frequency and colonization density of *Malassezia* spp were associated with the age and

sebaceous gland activity of the subjects in the studied area.^{3,22} The frequency and density of colonization of *Malassezia* appeared to increase around puberty, which can be correlated with the increase in sebaceous gland activity seen at this time. This study showed there was no significant difference between sexes in each age group and sites studied. Similar observation is also shown by other studies in Bosnia and Herzegovina as well in Panama.^{16,23} This is in contrast with a study on healthy Korean subjects.¹³

Being lipid dependent, they are commonly found to be distributed in areas rich in sebaceous glands, such as the chest, back, neck and shoulders. They reside in the stratum corneum and hair infundibulum, where they utilize sebum for their growth and maintenance. The triglycerides and esters present in the sebum are broken down into diglycerides, monoglycerides, and free fatty acids which provide nourishment for them.²⁴ According to different sites sampled, the positive rate of isolation of different *Malassezia* in culture was highest in chest with 200 positive cases out of 240 (83.33%), followed by back with 176/240 (73.33%) and neck 76/240 (31.67%). The colonization density of *Malassezia* was significantly higher in the chest (83.33%) compared with other areas ($P < 0.05$) reflecting the difference in skin lipid levels in different body areas. This was consistent with the studies done in normal Korean healthy men and women.²¹

The highest detection rates were shown by *M. globosa* (46.46%), *M. furfur* (25%), and *M. restricta* (22.57%), respectively. Other species like *M. sympodialis*, *M. slooffiae*, *M. obtusa* and *M. pachydermatis* were less frequently isolated. The highest detection rates of *M. globosa* is in agreement with studies from Iran Korea and Spain and stands in contrast with the other studies from India Canada and Russia in which they reported *M. sympodialis* as the commonest species, Bosnia and Herzegovina as *M. restricta* and Iran as *M. furfur* from normal healthy skin.^{15,14,25,19,12,16,18} The differences between this study and others may not only be due to the result of variation in species on the skin of individuals in different countries, but also by the difference in sampling methods i.e.; swabbing, a relatively insensitive method, scraping or adhesive transparent tape method, difference in culture media used (SDA with olive oil, mDA, LNA) and difference in incubation temperature of inoculated culture media.^{18,6}

CONCLUSION

The overall colonization rate of *Malassezia* spp in normal healthy individuals found in this study was 62.78%. The population density of *Malassezia* was significantly higher in the age group of 21–30 years. The colonization density of *Malassezia* was significantly higher in the chest compared with other areas. *M. globosa* was identified as the common species (46.46%) followed by *M. furfur* 25% and *M. restricta* 22.56% in the healthy human skin.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee School of Health Sciences, Kannur University

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Cite this article as: Remya VS, Arun B, Sheeba PM, Kokkayil P. A study on malassezia micro flora in the skin of healthy individuals in North Kerala, India. *Int J Res Med Sci* 2017;5:4600-3.