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ORIGINAL ARTICLE

Distribution of *Malassezia* species on healthy human skin in Bosnia and Herzegovina: correlation with body part, age and gender

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ABSTRACT

Background and Objectives: The genus *Malasezia* currently includes fourteen species that have been isolated from healthy and diseased human and animal skin. However, there were differences with respect to the species most commonly isolated, not only in patients with various skin diseases but also between healthy individuals. The aim of this study was to analyze the prevalence of *Malassezia* species from clinically normal skin of the scalp and trunk of healthy individuals and to examine if the range of species varies according to body site, gender and age.

Materials and Methods: The study was conducted at the Department of Dermatovenerology, University Clinical Center in Sarajevo, Bosnia and Herzegovina from December 2012 to May 2013. One hundred healthy men and women with no skin diseases and aged from <1 to 82 years were studied. The samples were obtained by scraping the skin surface from the upper and middle part of trunk and from scalps of all subjects and then incubated on modified Dixon agar. The yeasts isolated were identified by their morphological and physiological properties according to Guillot *et al.* method.

Results: *M. sympodialis* was the predominant species on trunk skin in older subjects, *M. restricta* on scalp skin in age groups 21-35 years, while *M. globosa* was identified as common species in adults (36-50 years), both from scalp skin and trunk skin. From the trunk skin *M. furfur* was the most frequent in children.

Conclusion: This study confirmed that cutaneous *Malassezia* microbiota in healthy subjects varies by body part and age but not by gender.

Keywords: *Malassezia*, species, identification, healthy skin

INTRODUCTION

Malassezia yeasts have been recognized for more than 150 years (1) as members of the normal human cutaneous commensal flora. However, under the influence of predisposing factors these lipolphilic yeasts may become pathogenic and associated with several diseases such as pityriasis versicolor, folliculitis, seborrheic dermatitis, confluent and reticulate papillomatosis, and even systemic infections (2,3).

The taxonomy of *Malassezia* has been confusing since their early description, because yeasts are dimorphic, existing in both yeast and mycelial phases (4). Today, the genus *Malassezia* includes 14 lipophilic species which have been identified with a conventional culture method or using molecular methods. Most human isolates belong to the species *M. globosa*, *M. restricta*, *M. sympodialis, M. furfur*, *M. slooffiae* (5), *M. dermatis* (6), *M. japonica* (7) and *M. yamotoensis* (8).

In humans *Malassezia* yeasts inhabit sebum-rich

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areas of the skin, including the trunk and the head region, with population densities peaking between 20-45 years (9). However, there is a great variation regarding presence and density in various skin locations (9-11), in children compared with adults (11-13) and in normal skin compared with diseased skin (2).

Although the role of *Malassezia* in the pathogenesis of cutaneous diseases is not fully understood, recent studies have shown that decreased density of *Malassezia* led to improvement of these diseases. There is also some controversy as to whether specific species cause different skin diseases (2,3).

Several studies have been carried out worldwide on the epidemiology of *Malassezia* species isolated from healthy individuals (11,14-24). The findings vary within the same body sites and same age groups depending on the researcher as different methodologies, isolation media and identification procedures have been employed. The difference in the results may also be attributable to the various internal and external factors such as racial and geographic differences and inevitable errors in each research. Therefore, it is important to recognize the existence of a difference in the distribution of *Malassezia* species in various ages and different body sites.

The aim of this study was to isolate and identify *Malassezia* species from the healthy human skin by using morphological, biochemical and physiological criteria and to examine if the range of species varies with different body areas, gender and age groups.

PATIENTS AND METHODS

Patients. The study was conducted from December 2012 till May 2013 at the Department of Dermatovenerology, University Clinical Center, Sarajevo, Bosnia and Herzegovina.

One hundred healthy individuals (50 females and 50 males; age range <1 to 82 years, mean 32) with no skin diseases and without any known underlying disease were included in the study. Subjects were selected among medical students (45), medical staff (20) and their children (17) or relatives (18).

All participants were instructed not to take a shower or use emollients on the day of investigation. Only those subjects who had not used any topical and oral treatment or ultraviolet phototherapy in previous two months were included in the study. All the subjects gave their written informed consent with the requirements of the Institutional Ethics Committee.

Samples. All samples consisted of scales and scrapings from the upper and middle part of trunk and from scalps in all participants. Collected samples were inoculated into Sabouraud dextrose agar and into modified Dixon agar (mDA) consisting of 3.6% malt extract, 0.6% mycological peptone, 2.0% desiccated ox bile (Sigma Chemical Co. Ltd, Dorset, UK), 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.05% cycloheximide, and 1.2% agar (pH 6.0). The medium was always used within one week of preparation and the cultures were inoculated at 32°C for seven days.

Identification of Malassezia yeasts. *Malassezia* species were identified according to their macroscopic and microscopic features and physiological characteristics. The macroscopic features of the predominant colonies included their shape, size, color, consistency, and the characteristics of medium around them. Microscopic features of the yeast cells in culture were described after lactophenol staining and included the predominant morphology, size and budding base of the yeasts.

To assess the physiological properties of the yeasts catalase reaction was determined by using a drop of hydrogen peroxide (30%) onto a culture smear on a glass slide. The production of gas bubbles, indicative of release of oxygen, was considered a positive reaction. Utilization of Tween compounds was done according to the test originally described by Guillot *et al.* (25) and later modified by Gupta *et al.* (26). Yeast suspension, obtained by inoculating 5 mL of sterile water with a loopful of actively growing yeasts, was inoculated on Sabouraud glucose agar. The inoculum was then spread evenly. Each plate was divided into four sections and 5 mL of Tween 20, 40, 60 and 80 were added into a hole made in center of each section and incubated for a week at 32°C. Utilization of Tweens was assessed by the degree of growth and/or reaction of the lipophilic yeasts around individual holes.

The ability of the various *Malassezia* species to growth on mDA at 38°C was studied. A sample of actively growing cultures was transfered to mDA and incubated at 38°C for 7 days after which the ability to grow was investigated.

The β-glucosidase activity (spliting of esculin)

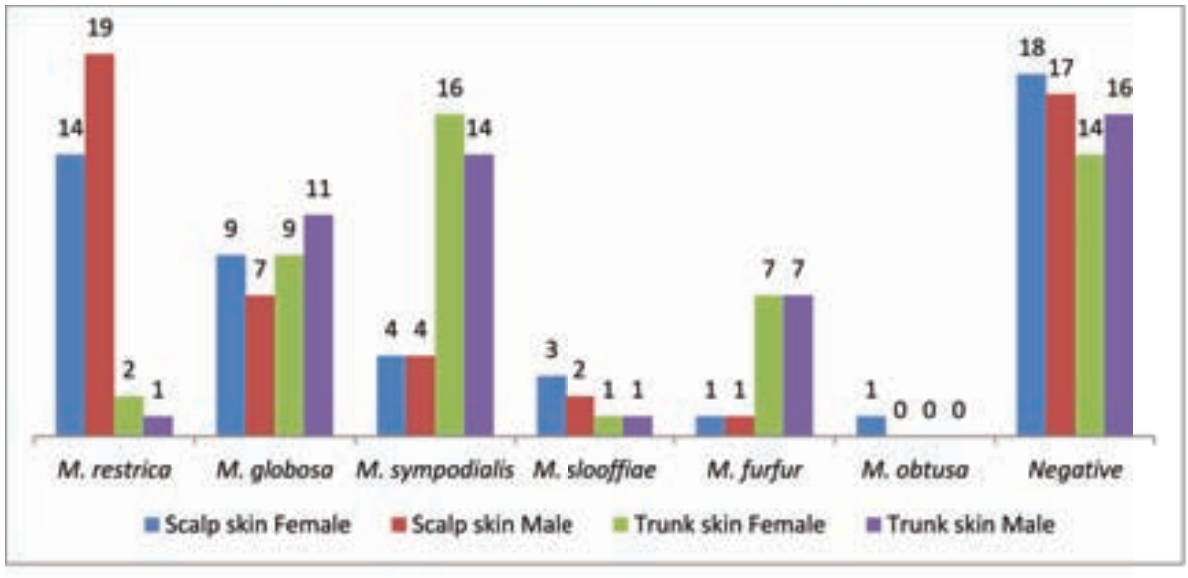

Fig 1. *Malassezia* species distribution from scalp and trunk skin of healthy subjects according to gender

of different *Malassezia* species was assayed using method described by Mayser *et al.* (27). Briefly, a loop of fresh yeast was inoculated deeply in the esculin agar tube and incubated for 5 days at 32°C. The splitting of esculin is revealed by darkening of the medium.

Statistics. Chi-squared test with Yates' correction for a small sample size was carried out to determine the statistical significance of differences in proportions. We used a statistical software package Minitab 13.0. Significance level was set at $P < 0.05$.

RESULTS

Cultures. *Malassezia* yeasts were found in 65% samples taken from healthy scalp skin. The most frequently isolated species was *M. restricta* found in 33 patients, followed by *M. globosa* (16%) *M. sympodialis* (8%), *M. slooffiae* (5%), *M. furfur* (2%) and *M. obtusa* in one case.

The results of culture obtained from healthy trunk skin were positive for *Malassezia* yeasts in 69 cases. The predominant species was *M. sympodialis* found in 30 patients and the prevalence of other species was 20% for *M. globosa* and 14% for *M. furfur.* Other species were less frequently isolated: *M. restricta* 3%, *M. slooffiae* 2%.

Statistically significant differences were found in the distribution of the species isolated from healthy scalp and trunk skin – *M. restricta* was more commonly positive in healthy scalp skin cultures (ratio 11.0), while *M. furfur* and *M. sympodialis* were more frequent in the healthy trunk skin cultures (ratio 7 and 3.7, respectively) ($P < 0.05$) (Table 1).

Distribution of Malassezia species isolated according to demographic parameters

Gender. The same numbers were woman and man (50%). No statistically significant differences were found between the genders in the species isolated from scalp and trunk skin ($P > 0.05$) (Fig. 1).

Age. Subjects were grouped according to age as follows: 0-10 (15%), 11-20 (19%), 21-35 (30%), 36-50 (20%), and ≥51 years of age (16%).

Site of infection. Among *Malassezia* species cultured from scalp skin, *M. restricta* predominated in age groups 21-35 (39%) and 11-20 (33%). *M. globosa* was identified as common species in adults (36-50 years), both from scalp skin and trunk skin, in a frequency of 56% and 50%, respectively. From the trunk skin, *M. sympodialis* was most frequently found in older subjects (>51 years) – 30%, while *M. furfur* was the most predominant in the group of less than 10 years – 50 (Fig. 2).

Statistically significant differences were observed in the distribution of the species isolated from scalp and trunk skin according to the age groups ($P < 0.05$).

DISCUSSION

The *Malassezia* genus contains a group of lipophilic yeasts that form part of the normal human skin.

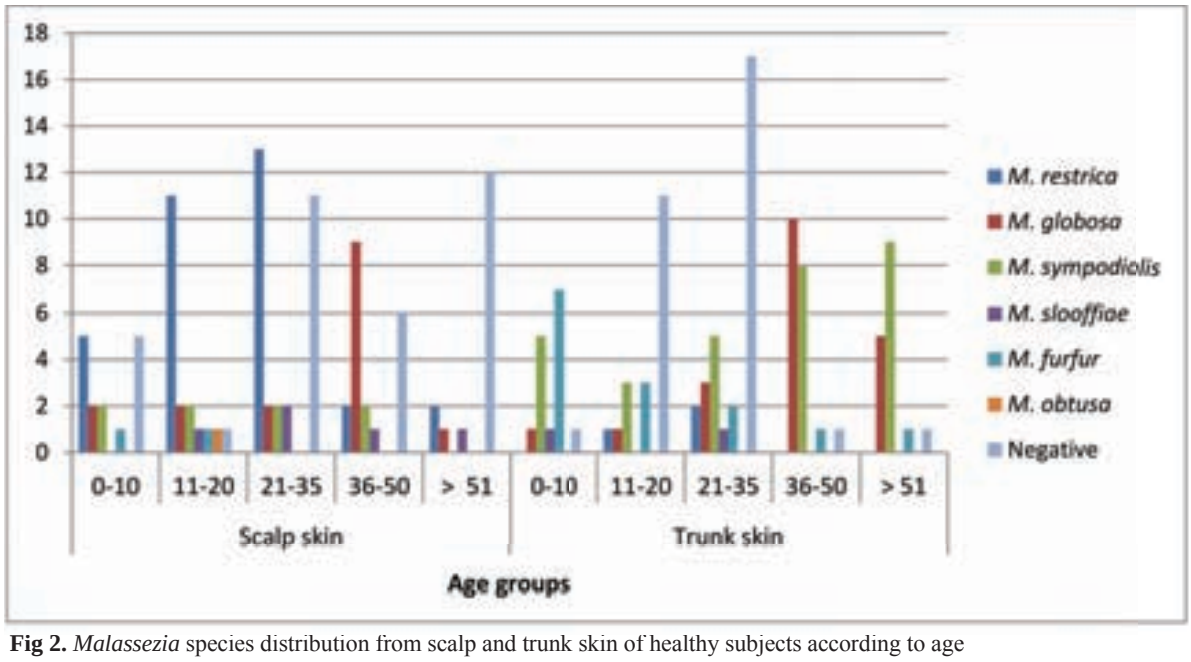

Fig 2. *Malassezia* species distribution from scalp and trunk skin of healthy subjects according to age

The colony-formation begins immediately after the birth and increases significantly with age of the neonate. It has been reported that skin colonization by *Malassezia* yeasts was 5% at the first week and 30% at 2-4 weeks of life (28). Using a molecular analysis, Japanese investigators were able to detect *Malassezia* from 89% and 100% of neonate samples on days 0 and 1 after birth, respectively. Subsequently, the level of *Malassezia* colonization of the neonates increased with time, whereas that of the mothers did not change (29). Generally, the carriage of *Malassezia* is the highest during puberty, which is related to the increase in sebaceous gland activity seen at this time.

In one study from Venezuela, 65% cultures yielded positive specimens for *Malassezia* (30), while earlier studies have identified even greater carriage rates of *Malassezia* of healthy pubertal children: 74% on the scalp (31), 93% on the back (32), and 87% on the forehead (33).

In adults, the frequency and density of colonization is related to the age and to the activity of the sebaceous glands in the area studied. The highest population densities were noted from chest, upper back and forehead and men yielded more yeasts in lower back and thigh than women (9). Bergbrant and Faergemann found that the density of *Malassezia* species on the skin decreased with increasing age, which was probably due to a reduction in the level of lipid on the skin (34).

Several studies have been carried out world-wide on the distribution of the newly defined species of *Malassezia* on healthy adult human skin in which variable results have been reported from different geographical regions (11,14-24) (Table 2).

In general, it seems that the most common *Malassezia* species cultured from healthy scalp skin is *M. restricta* (11,16), while *M. globosa* and *M. sympodilais* are two most frequently isolated species from the trunk (11,14,15,17-24). Other species are less common, but not completely absent.

Similar to the majority of other investigations, we found *M. sympodialis* as the most frequent species on normal trunk skin isolated in 30% of cases. This species emerges as the predominant species on healthy skin, especially on the trunk, where it can be recovered in great numbers in more than 50% of individuals (18,20).

By contrast, *M. globosa* has been reported as the most frequently isolated species in some other investigations (15,17,19,21-23). In our study, *M. globosa* is less common species found in 20% of healthy individuals which goes well with the studies of Gupta *et al.* (18) and Kaur *et al*. (24). This species is reported to be a causative agent of pityriasis versicolor, found in filamentous form in the scales from this skin disorder (14,15,17,19,21,35).

M. furfur was isolated in 14% and this species is found to be less common inhabitant of healthy skin (11,17,19,21). However, *M. furfur* is confirmed to also

Table 1. *Malassezia* species isolated from scalp and trunk of healthy subjects

No	Age (years)	Gender	Isolated species	
			Scalp	Trunk
1.	0 (10 months)	F	Negative	*M. furfur*
2.	2	F	*M. restricta*	Negative
3.	3	F	*M. restricta*	*M. globosa*
4.	5	M	*M. restricta*	*M. sympodialis*
5.	6	M	*M. restricta*	*M. slooffiae*
6.	6	F	*M. restricta*	*M. sympodialis*
7.	7	M	Negative	*M. sympodialis*
8.	9	F	Negative	*M. furfur*
9.	9	F	Negative	*M. sympodialis*
10.	9	F	Negative	*M. furfur*
11.	10	F	*M. globosa*	*M. sympodialis*
12.	10	F	*M. globosa*	*M. furfur*
13.	10	F	*M. sympodialis*	*M. furfur*
14.	10	F	*M. sympodialis*	*M. furfur*
15.	10	F	*M. furfur*	*M. furfur*
16.	11	M	*M. restricta*	*M. sympodialis*
17.	13	F	Negative	Negative
18.	15	F	*M. restricta*	Negative
19.	16	F	*M. restricta*	Negative
20.	16	F	*M. globosa*	Negative
21.	17	M	*M. restricta*	*M. restricta*
22.	18	F	*M. restricta*	*M. resticta*
23.	18	M	*M. sympodialis*	*M. furfur*
24.	19	M	*M. restricta*	Negative
25.	19	M	*M. sympodialis*	Negative
26.	19	M	*M. restricta*	*M. sympodialis*
27.	19	F	*M. globosa*	*M. globosa*
28.	19	F	*M. restricta*	Negative
29.	20	F	*M. slooffiae*	Negative
30.	20	M	*M. restricta*	Negative
31.	20	M	*M. furfur*	*M. furfur*
32.	20	M	*M. restricta*	*M. furfur*
33.	20	F	*M. obtusa*	Negative
34.	20	M	*M. restricta*	*M. sympodialis*
35.	21	F	*M. restricta*	Negative
36.	21	F	*M. restricta*	Negative
37.	21	M	*M. globosa*	Negative
38.	23	M	*M. restricta*	Negative
39.	23	F	Negative	*M. sympodialis*
40.	23	M	*M. restricta*	Negative
41.	24	M	Negative	*M. sympodialis*
42.	24	F	*M. restricta*	Negative

Table 1. Continued

No	Age (years)	Gender	Isolated species	
			Scalp	Trunk
43.	24	F	Negative	*M. slooffiae*
44.	24	M	Negative	Negative
45.	24	F	*M. restricta*	Negative
46.	25	M	Negative	*M. furfur*
47.	25	F	*M. restricta*	Negative
48.	25	M	*M. restricta*	*M. sympodialis*
49.	25	M	*M. restricta*	*M. furfur*
50.	26	F	*M. globosa*	*M. globosa*
51.	26	M	*M. restricta*	*M. restricta*
52.	26	M	*M. slooffiae*	Negative
53.	27	M	Negative	Negative
54.	28	M	*M. restricta*	*M. globosa*
55.	28	M	Negative	Negative
56.	28	M	*M. slooffiae*	Negative
57.	29	M	Negative	*M. globosa*
58.	29	M	*M. restricta*	Negative
59.	30	F	Negative	*M. restricta*
60.	31	M	*M. sympodialis*	Negative
61.	32	M	Negative	*M. furfur*
62.	33	M	*M. sympodialis*	Negative
63.	35	F	*M. restricta*	*M. sympodialis*
64.	35	M	Negative	Negative
65.	36	M	*M. restricta*	*M. globosa*
66.	37	M	*M. globosa*	*M. globosa*
67.	37	F	*M. globosa*	*M. sympodialis*
68.	38	F	*M. slooffiae*	*M. sympodialis*
69.	39	M	*M. globosa*	*M. globosa*
70.	39	F	Negative	*M. sympodialis*
71.	40	M	*M. globosa*	*M. globosa*
72.	40	F	*M. sympodialis*	*M. globosa*
73.	41	F	Negative	*M. sympodialis*
74.	42	F	Negative	*M. globosa*
75.	43	F	*M. sympodialis*	*M. sympodialis*
76.	44	F	*M. globosa*	*M. sympodialis*
77.	45	M	*M. globosa*	*M. globosa*
78.	47	F	Negative	*M. globosa*
79.	48	M	*M. globosa*	*M. globosa*
80.	49	F	Negative	Negative
81.	49	M	Negative	*M. sympodialis*
82.	50	F	*M. globosa*	*M. sympodialis*
83.	50	F	*M. globosa*	*M. sympodialis*
84.	50	M	*M. restricta*	*M. globosa*
85.	51	M	*M. restricta*	*M. sympodialis*

Table 1. Continued

No	Age (years)	Gender	Isolated species	
			Scalp	Trunk
86.	54	F	Negative	*M. globosa*
87.	56	M	Negative	*M. globosa*
88.	59	F	Negative	*M. sympodialis*
89.	59	F	Negative	Negative
90.	61	M	Negative	*M. sympodialis*
91.	66	M	Negative	*M. furfur*
92.	66	M	*M. restricta*	*M. sympodialis*
93.	67	M	Negative	*M. globosa*
94.	67	M	Negative	*M. sympodialis*
95.	68	M	Negative	*M. sympodialis*
96.	70	F	*M. slooffiae*	*M. sympodialis*
97.	74	F	Negative	*M. globosa*
98.	78	F	Negative	*M. globosa*
99.	80	M	*M. globosa*	*M. sympodialis*
100.	82	F	Negative	*M. sympodialis*

be responsible for pityriasis versicolor, particularly under tropical climate (36).

In contrast to healthy trunk skin, *M. sympodialis* was recovered less frequently from the scalp skin of same subjects (8%), whereas *M. restricta* was the commonest species (33%). This species is isolated regularly from the scalp and face of patients with seborrheic dermatitis (5,14,22). Oh *et al.* in Korea also found *M. restricta* to be particularly associated with scalp and *M. globosa* and *M. sympodialis* with the chest, whereas other species (*M. furfur, M. obtusa* and *M. slooffiae)* were less frequently recovered (23).

In our study, *M. slooffiae* and *M. obtusa* were isolated only from a few samples taken from our subjects. These species are considered to be very rare on healthy skin (20,24) and only infrequently isolated from the cases of pityriasis versicolor (17,35), atopic dermatitis (15,20,26) and seborrheic dermatitis (14,20).

M. pachydermatis was not recovered from any of our samples either from trunk or from healthy scalp skin. This species is confirmed to be clearly adapted to animals, although it has been involved in some systemic human infection. The presence of this species on human skin is rare and transient, occurring possibly by transmission from animal pets and environmental sources (37).

Several studies demonstrated that the distribution of *Malassezia* species on head and trunk region is parallel with the density and activity of pilosebaceous glands in these areas. The difference in species according to different body areas is presumably to be attributed to lipid content and different lipid components in each body area (11).

The higher detection rate of *Malassezia* species was observed using molecular determination method than by conventional culture methods. These variations may be attributed to the different sampling technique and inadequate determination of the relative proportion of species on the skin, or the consent ability of the fungus to grow in each specified medium that have impact on the range of species recovered (20).

A correlation between the prevalence of *Malassezia* species and gender of the subjects has been observed without differences among the woman and man. However, statistical difference was noted among age groups and isolated species. *M. globosa* was the predominant species in adult subjects regardless of body part, *M. restricta* was recovered more frequently from the scalp skin in the teens and young adults, while on the trunk sin *M. sympodialis* was most frequently found in older subjects. Our results are concordant with the majority of studies worldwide which clarified that the cutaneous *Malassezia* microbiota of healthy subjects differed by age (11,13,38). This was contrary to observation of Gupta *et al.* who cultured more frequently *M. globosa* on younger subjects and *M. sympodialis* on the skin of adolescents and adults (18).

In conclusion, researches recovered not only

Table 2. Results from epidemiological studies of *Malassezia* species isolated from scalp and trunk skin of healthy normal subjects

Country	Sampling method	Culture medium/ Identification method	Site sampled	Number of samples/ pos cultures	Species recovered (% of samples)	Ref.
Spain	Scraping	mDixons/ By Guillot *et al.* (1996)	Trunk (shoulders)	75/52	*M. sympodialis* (96) *M. slooffiae* (4)	14
Japan	Swabing	mDixons/ By Guillot *et al.* (1996)	Scalp	35/5	*M. globosa* (6) *M. sympodialis, M. furfur, M. slooffiae* (3 each)	15
			Trunk	35/24	*M. globosa* (51) *M. sympodialis* (26)	
Japan	Tape strip	None (direct DNA extraction)/ Molecular-Nested PCR	Scalp	18/14	*M. restricta* (61) *M. sympodialis* (50) *M. globosa* (44)	16
Iran	Tape strip	mDixons/ By Guillot *et al.* (1996)	Not stated	100/60	*M. globosa* (42) *M. sympodialis* (25) *M. furfur* (23)	17
Canada	Contact plates	LNA/ By Guillot *et al.* (1996)	MS	245/172	*M. sympodialis* (57) *M. globosa* (32)	18
Tunis	Swabing	mDixons/ By Guillot *et al.* (1996)	Trunk (chest)	30/9	*M. globosa* + *M. sympodialis* (10) *M. furfur, M. sympodialis* (7 each)	19
Sweden	Contact plates	LNA/ By Guillot *et al.* (1996)	Trunk (upper back)	31/26	*M. sympodialis* (69) *M. obtusa* (15) *M. globosa* (12)	20
Korea	Scrub-wash	LNA/ By Guillot *et al.* (1996)	Scalp	120/56	*M. restricta* (39) *M. globosa* (22.5) *M. slooffiae* (1.5)	11
			Chest	120/101	*M. globosa* (49) *M. restricta* (21) *M. sympodialis* (10)	
Iran	Scraping	LNA/ Molecular – PCR-FLPM	Trunk	95/94	*M. globosa* (70) *M. furfur* (15) *M. sympodialis* (6)	21
Japan	Tape strip	None (direct DNA extraction)/ Molecular-Nested PCR	Face	30/30	*M. globosa* (87) *M. restricta* (83) *M. sympodialis* (37)	22
Korea	Scrub-wash	LNA/ Molecular-Nested PCR	MS	110/70	*M. globosa* (32) *M. restricta* (30) *M. sympodialis* (15)	23
India	Scraping	mDixons/ By Guillot *et al.* (1996)	Trunk (upper back)	45/21	*M. sympodialis* (48) *M. obtusa* (19) *M. globosa* (14)	24

mDixons, modified Dixon agar; LNA, Leeming and Notman agar; FLPA, fragment length polymorphism analysis; PCR, polymerase chain reaction; MS, multiple sites

different species distributed on the same anatomic area, but also the same species in even the same frequency from different body areas. These facts suggest that besides different sampling methods and culture media used, there are probably some internal and external factors as well as geographical and ethnic variations reflected in the predominance of different species on the skin of individuals in different parts of the world.

New molecular approaches to the identification of different species will certainly answer many outstanding questions associated with *Malassezia* species and their role in human pathophysiology.

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