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Seroprevalence of *Malassezia furfur* in an urban population in Malaysia

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Abstract

An in-house prepared *M. furfur* antigen was used to carry out a seroprevalence study in an urban population in Malaysia by indirect immunofluorescence assay. Of the 800 serum samples from all ages screened, 738 samples were positive for *M. furfur* specific IgG, giving an overall seropositive rate of 92.3%. There was no significant difference in the seropositive rates among the different gender group and races. However, there was a statistical significant difference in the seropositive rate among different age groups with a lower rate (73%) for the age group 5 years old and below, which increased rapidly to 99% for the 16 to 20 years old age group but declined slightly for the oldest age group.

The degree of seropositivity, which semi-quantitatively reflect the anti-*M. furfur* specific IgG titre, did not show any significant difference among the gender and racial groups. On the other hand, there was a significant difference in the degree of seropositivity among the various age groups, with the 16 to 20 years old age group having the highest antibody titre and the extreme of age groups having the lower antibody titre.

Key words: *Malassezia furfur*, seroprevalence

INTRODUCTION

The genus *Malassezia* comprises lipophilic yeasts. They are considered as the normal cutaneous microbiota of humans and some warm-blooded animals.[1] However, under certain circumstances, these yeasts are associated with a variety of diseases in both humans and animals.[2-6] They were implicated as the aetiological agents of systemic infection especially in patients on intralipid therapy, as well as of a number of skin disorders such as pityriasis versicolor, seborrheic dermatitis, dandruff and Malassezia folliculitis.[2-9]

The taxonomy of the yeast has undergone many changes since its first detection and implication as the cause of pityriasis versicolor by Eichstedt in 1846.[10] In 1953, Robin named the fungus as *Microsporum furfur* which he thought was related to the dermatophyte *Microsporum audouinii*.[10] In 1874, Malassez described this yeast from a lesion of the scalp as "spores". Subsequently, various names had been used to describe this yeast-like organism such as *Pityrosporum (P) ovale*, *Saccharomyces ovale*, *P. orbiculare*.[10] In 1889, Billion created the new genus *Malassezia* to accommodate this microorganism.[10] The lipophilic nature of *Malassezia* organisms was described in 1939 by Benham.[11] The great micromorphological polymorphism and the lack of suitable methods for isolation in the early period were the main reasons that made their study and classification difficult.[12] The genus has recently been revised based on the morphology, ultrastructure, physiology and molecular biology of these yeasts and presently expanded to comprise seven species: *M. pachydermatis*, *M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtuse*, *M. restricta*, and *M. slooffiae*.[13,14] With the exception of *M pachydermatis*, the remaining six species are lipid-dependent yeasts, because of their requirement for long chain fatty acids for in-vitro growth.[15-17]

The yeast is found on the stratum corneum of the epidermis and in the piliferous follicles. It can stimulate an immune response that has been documented in healthy subjects as well as those affected by associated dermatological diseases.[18-24] The aim of this study was to determine the antibody response in an urban population at various age groups and to determine any correlation with its variation of isolation in the various age groups reported in the literature.[25-28]

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MATERIALS AND METHODS

Antigen preparation

The *M. furfur* yeasts previously isolated using Sabouraud Dextrose Agar supplemented with olive oil from a patient with skin disease was used to prepare the required antigen for the seroprevalence study. The culture of *M. furfur* yeast was carried by streaking the organism onto an in-house prepared culture medium, IMU-mf agar, and a single colony was selected after 48 hours of culture at 35 °C. The identity of the yeast derived from the selected single colony was confirmed as *M. furfur* by its morphology, lipid dependency for growth and molecular characterization. In molecular characterization, the yeast DNA was extracted following the method described by Makimura *et al*.[29] and the DNA sequence of nuclear ribosomal internal transcribed spacer 1 region was amplified and sequenced using the primer pair described in Makimura *et al*.[30] Following the confirmation of the identity of the organism as *M. furfur*, the scale-up growth of the selected single colony of yeast was carried out by spreading a suspension of the yeast in sterile phosphate buffered saline (PBS) on the surface of the IMU-mf agar. After 48 hours of cultivation at 35 °C, the yeast cells were harvested and suspended in 10 ml of PBS containing 5% of Triton-X for 10 minutes with intermittent agitation to reduce the extent of aggregation. The yeast cells were then washed four times to remove the Triton-X using sterile PBS by centrifugation at 1000 g for 10 minutes in each wash. After the last wash, the yeast cells were suspended in sterile PBS at a concentration of 500 cells per millilitre. Ten microlitre of the resultant yeast suspension was carefully layered onto each well of the Teflon coated slides. The slides were air-dried over a warm plate and subsequently fixed in cold acetone for 10 minutes. The Teflon slides containing the fixed yeast cells antigen were stored at -20 °C until used.

Serum samples

Serum samples frozen at –20 °C kept in the Virology Unit, Department of Medical Microbiology, University of Malaya collected between January 1999 to December 2000 from the following categories of population were included in the serosurvey:

i) healthy donors of platelets and potential organ donors (inclusive of bone marrow);
ii) Undergraduate students seen in the student health clinic of the University of Malaya, Kuala Lumpur; and
iii) Patients treated in the University Hospital, Kuala Lumpur for various surgical, obstetrical, medical, opthalmological and ENT conditions.

Patients on any form of immunosuppressive therapy or with any form of neoplastic, immunosuppressive or autoimmune diseases were excluded. The study population, selected randomly and consisting of various races, was divided into eight age groups. Each group consisted of 50 males and 50 females. A total of 800 serum samples were screened for the presence of *M. furfur* specific IgG.

Procedure of testing

The acetone-fixed whole yeast cell antigen prepared earlier on the Teflon coated slide was used to detect the present of *M. furfur* specific IgG at 1:25 dilution by indirect immuno-fluorescent assay. Two hundred serum samples were tested in each batch. Each batch test run included one blank (PBS) control, one negative serum control and four positive sera each with 1, 2, 3 and 4 degree of seropositivity. The degree of seropositivity was graded by the estimated percentage of yeast cells in the well with positive "apple-green" fluorescence and the intensity of fluorescence to reflect the antibody titre. The criteria of grading for each category was as follows:

0: negative;
1: at least 70% of yeast cells with discernable fluorescence;
2: almost all yeast cells with obvious fluorescence;
3: all yeast cells with strong fluorescence; and
4: all yeast cells with strong brilliant fluorescence.

Ten random serum samples of each category were subsequently serially titrated by doubling dilution and each diluted sample was tested by similar test system to assay the antibody titre. All the results of the test were read and agreed upon by the same two readers.

Briefly, 5 µl of thawed serum was diluted with 120 µl of sterile PBS. 20 µl of each diluted serum sample was transferred onto each well of the Teflon coated slide. The slides with the applied test sera were incubated in a moist chamber for 30 minutes at 37 °C. They were initially rinsed off with PBS before being subsequently soaked for a further 10 minutes in PBS solution kept in gentle motion by magnetic stirrer. The slides were allowed to air dry over a

warm plate and probed with 20 µl of 1:40 diluted fluorescein conjugated rabbit anti-human IgG (Dako, USA). The slides were then incubated for another 30 minutes at 37 °C in a moist chamber. The same process of washing and drying was again carried after incubation. Following this, the slides were mounted with a commercially supplied mounting fluid and examined under a UV fluorescence microscope (Olympus BX50, Japan) at 400X magnification.

Statistical analysis

Statistical analysis was performed using two statistical software programmes (MINITAB and Instat). The results of the study were subjected to chi-square test for any statistical significant association. A p-value of 0.05 or less was taken as the level of significant association for each ordinal variable with the relevant adjusting variables.

RESULT

In this study, the grading criteria that semi-quantitatively reflected the titre of anti-*M furfur* IgG for each category as described previously was shown in Figure 1. The titre of anti-*M furfur* specific IgG based on 10 random serum samples of each category was 1:25 to 1:50 dilution for Category 1, 1:100 dilution for Category 2, 1:200 dilution for Category 3 and 1:400 to 1:800 dilution for Category 4. Anti-*M. furfur* serum raised in rabbit by immunization using whole *M. furfur* yeast as antigen reacted only with fixed yeast cells of *M. furfur* used in this study and the same serum did not cross-react with similar preparations of yeast cells of *C.* (*Candida*) *albicans*, *C. tropicalis*, *C. parasilopsis*, *Geotrichum*, *Rhodutorula rubrae, Penicillium marnaeffei* (data not shown).

The distribution of patients by race and gender in each age group was shown in Table 1. There was a slight skewed inclusion of Chinese especially males in the older adult age groups (31 yr and above) while it was a reverse for the Malay racial group. The age group, 51 years old and above consisted of patients with ages ranged from 51 to 81 years old with a mean of 58.9.

Of the 800 serum samples from all age groups tested, 738 samples were positive for *M. furfur* specific IgG, giving an overall seropositive rate

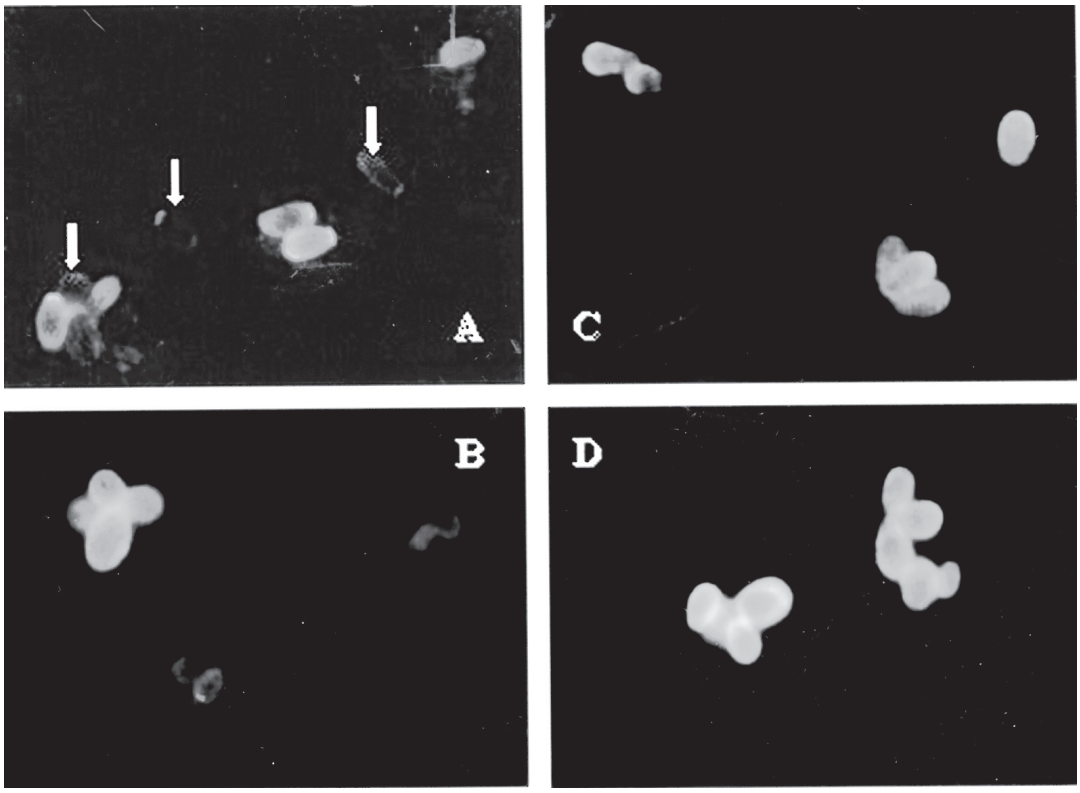

FIG. 1: A composite photograph demonstrating the level of human serum IgG against *M. furfur* yeasts by indirect immunofluorescence. The degree of seropositivity for Categories 1, 2, 3, and 4 are represented by A, B, C and D respectively. Arrows indicate yeast cells with negative fluorescence. Magnification: 1000X.

Table 1: Distribution of patients by race in each age and gender groups.

	Chinese		Malays		Indians		Others	
Age-group (year)	Male	Female	Male	Female	Male	Female	Male	Female
0.5 – 5	21	12	18	25	8	12	3	1
6 – 10	14	19	27	20	7	11	2	0
11 – 15	15	9	21	22	14	18	0	1
16 – 20	24	14	18	24	5	10	3	2
21 – 30	27	13	12	29	8	8	3	0
31 – 40	26	19	10	23	13	7	1	1
41 – 50	23	25	10	16	16	9	1	0
51 and above	33	33	7	7	10	10	0	0
Total	183	144	123	166	81	85	13	5

Table 2: The seropositive rates of *Malassezia furfur* by gender and race.

Variable	Group	No. tested	No. positive (%)	$\chi 2$	p-value
Gender	Male	400	367 (91.8)		
	Female	400	371 (92.8)		
	Total	800	738 (92.3)	0.280	0.597
Race	Chinese	327	308 (94.2)		
	Malays	289	264 (91.3)		
	Indians	166	149 (89.8)		
	Others	18	16 (88.9)		
	Total	800	738(92.3)	3.702	0.296

Table 3: The seropositive rates of *M. furfur* by age-group.

Age-group	Male		Female		Total		$\chi 2$	p-value
(year)	No. tested	No. +ve (%)*	No. tested	No. +ve (%)	No. tested	No. +ve		
>0.5 – 5	50	35 (70)	50	38 (76)	100	73	0.203	0.652
6 – 10	50	43 (86)	50	46 (92)	100	89	0.409	0.523
11 – 15	50	46 (92)	50	47 (94)	100	93	Fisher	1.0000
16 – 20	50	50 (100)	50	49 (98)	100	99	Fisher	1.0000
21 – 30	50	48 (96)	50	49 (98)	100	97	Fisher	1.0000
31 – 40	50	49 (98)	50	49 (98)	100	98	Fisher	1.0000
41 – 50	50	49 (98)	50	47 (94)	100	96	Fisher	0.6173
51 and above	50	47 (94)	50	46 (92)	100	93	Fisher	1.0000

No. +ve (%)* = Number of positive (percent positive)

Table 4: The seropositive rate of *M. furfur* in each racial-group with respect to gender.

	Male		Female	
	No. tested	No. positive (%)	No. tested	No. positive (%)
Chinese	183	175 (95.6)	144	133 (92.4)
Malays	123	107 (87.0)	166	158 (95.2)
Indians	81	74 (91.4)	85	75 (88.2)
Others	13	11 (84.6)	5	5 (100)
Statistic	$\chi2 = 8.206$, p = 0.042		$\chi2 = 3.486$, p = 0.175	

Table 5: The degree of positivity of patients' sera by gender and racial groups.

Variable	Group	Degree of positivity				
		0	1	2	3	4
Gender	Male	33	77	142	114	34
	Female	29	68	152	119	32
Race	Chinese	24	49	106	86	24
	Malays	19	60	130	94	24
	Indians	17	36	49	48	16
	Others	2	0	9	5	2

Table 6: The degree of positivity of patients' sera by age groups.

Age-group (year)	Degree of positivity				
	0	1	2	3	4
0.5 – 5	27	25	34	12	2
6 – 10	11	16	31	35	7
11 – 15	7	17	44	29	3
16 – 20	1	4	27	41	27
21 – 30	3	8	29	44	16
31 – 40	2	15	42	35	6
41 – 50	4	25	43	24	4
51 and above	7	35	44	13	1

of 92.3%. The seropositive rates for males and females were 91.8% (367/400) and 92.8% (371/400) respectively (Table 2). There was no significant gender difference in seropositive rate ($\chi2 = 0.280$, df = 1, p = 0.597). The overall *M. furfur* seropositive rate among Chinese, Malays, Indians and other races were 94.2% (308/327), 91.3 (264/289), 89.8% (149/166) and 88.9% (16/18) respectively (Table 2). There was also no significant difference in the *M. furfur* seropositive rate among the races ($\chi2 = 3.702$, df = 3, p = 0.296).

The seropositive rates for each of the eight age groups are shown in Table 3. The seropositive rate was lowest in the age group below 5 years old (73%), increased rapidly to 99% in the 16 to 20 years old age group but declined slightly in the oldest age group (93%). There was a statistical significant difference in the seropositive rate among different age groups in this study ($\chi2 = 69.587$, df = 7, $p < 0.0001$). As with the finding in the overall seropositive rate among different sexes, there was also no significant gender

difference in the seropositive rate within each group (Table 3). Although there was no significant difference in the overall seropositive rate among races (Table 2) and racial groups within the female category (Table 4), there was a slight statistical significant difference for racial groups within the male category ($\chi 2 = 8.206$, df = 3, p = 0.042) (Table 4).

Among the 738 seropositive sera, the distribution in the Category 1, 2, 3, and 4 degree of seropositivity were 145, 294, 233 and 66 respectively. The distribution in each category degree of seropositivity with respect to gender and race are shown in Table 5. There was no significant difference in the degree of seropositivity among various gender and racial groups ($\chi 2 = 1.045$, df =3, p = 0.790; $\chi 2 = 9.615$, df = 9, p = 0.383). However, there was a significant difference in the degree seropositivity with respect to various age groups ($\chi 2 = 142.85$, df = 21, p < 0.0001). The 16 to 20 years old age group had the highest number of Category 4 degree of seropositivity with age group 21 to 30 years old had the second highest. Both had the highest number of Category 3 degree of seropositivity too (Table 6). Extremes of age had the lowest number of Category 4 degree of seropositivity but had the highest number of Category 1 degree of seropositivity (Table 6).

DISCUSSION

M. furfur is considered part of the normal microorganism flora of human skin though under certain circumstances it is known to cause a number of diseases. Because of its lipophillic and lipid dependency nature, it thrives better on the stratum corneum with higher level of human lipids in the form of sebum (cholesterol, sterol esters, squalene, triglycerides and free fatty acids). The sebum excretion rate is low in humans aged between birth and 10 years and increases between 11 and 15 years, reaching a maximum between the ages of 16 and 40 years and thereafter it declines.[31] In addition, sebum excretion differs according to the cutaneous area, which is higher on the forehead and trunk than on the upper and lower limbs.[32] Probably for these reasons, *M furfur* is not as frequently isolated in young children, from skin areas with low sebum excretion and in older persons aged over 50 years, unless in cases of frequent topical application of oil.[25-28]

A number of previous studies have described the presence of *M. furfur* specific antibodies in both healthy persons as well as those with diseases associated with the yeast using techniques such as enzyme-linked immunosorbent assay (ELISA), Western blotting, indirect immunofluorescence and immuno-electroprecipitation.[18-24] The present study adopted the indirect immunofluorescence assay technique using in-house prepared antigen to detect and semi-quantitate the anti-*M furfur* specific IgG. The results of this study showed that the seropositive rate corresponds to the age groups with the higher frequency of isolation of this yeast and the higher quantity of sebum presence on the skin of these age groups reported in other studies.[25, 26, 31] The degree of seropositivity, that is to semi-quantitatively reflect the titre of anti-*M. furfur* specific IgG, also tallied with the ages having the highest level of sebum excretion as reported by Cotterill *et al*.[31] Though the yeast is considered as a normal cutanous flora, the increased antibody response with respect to its increased multiplication in the presence of increased excreted sebum at specific ages may reflect its pathogenic potential. Its potential to cause other human diseases need further investigations especially in the age group with increased sebum excretion and also its causal relationship in diseases associated with increased sebum production such as severe acne.

In this study, a high seropositive rate (73%) was detected even in ages below 5 years old, which is not in concordance with the finding by Faggi *et al*. who reported zero positive rate for 100 serum samples derived from the age group below 10 years old.[24] Furthermore, the seropositive rates of various age groups in our study are also much higher than the seropositive rate of each respective age group in the Faggi *et al*. study.[24] The difference in the seropositive rates in comparison with the Faggi *et al*. study is mostly likely due to the different test systems used to perform the seroprevalence study. However, it does not exclude the possibility that it may actually reflect the different seropositive rates seen in different geographical locale. In concordance with the Faggi *et al*. study, there was no significant gender difference in the seropositive rate. The finding of this study showed that there was a slight significant difference for racial groups within the male category (Table 4) though there was no statistical significant difference in the overall seropositive rate among various races. This seemingly higher seropositive rate among the Chinese males (95.6% as shown in Table 4) may not reflect the true rate. This was attributed to the unintentional

inclusion of more adult Chinese males with ages 41 and above which had a higher seropositive rate and less Chinese males in the age group below 5 years old which carried lower seropositive rate (Table 1).

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REFERENCES

1. Midgley G. The diversity of *Pityrosporum* (*Malassezia*) yeasts in vivo and in vitro. Mycopathologia 1989; 106: 143-53.
2. Marcon MJ, Powell DA. Human infections due to *Malassezia* species. J Clin Microbiol 1992; 5: 101-19.
3. Gueho E, Boekhout T, Ashbee HR, Guilot J, van Belkum A, Faergemann J. The role of *Malassezia* species in the ecology of human skin and as pathogens. Med Mycol 1998; 36: 220-9.
4. Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborroeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. Med Mycol 2000; 38: 337-41.
5. Gupta AK, Kohli Y, Faergemann J, Summerbell RC. Epidemiology of *Malassezia* yeasts associated with pityriasis versicolor in Ontario, Canada Med Mycol 2001; 39: 199-206.
6. Brooks R, Brown L. Systemic infection with *Malassezia furfur* in an adult receiving long-term hyperalimentation therapy. J Infect Dis 1987; 156: 410-1.
7. Wurtz RM, Knospe WN. Malassezia fungemia in a patient without the usual risk factors. Ann Intern Med 1988; 109: 432-3.
8. Shek YH, Tucker MC, Viciana AL, Manz HJ, Connor DH. *Malassezia furfur*-Disseminated infection in premature infants. Am J Clin Pathol 1989; 92: 595-603.
9. Gueho E, Boekhout T, Ashbee HR, Guilot J. Association of *Malssezia pachydermatis* with systemic infections. J Clin Microbiol 1987; 25: 1789-90.
10. Kwon-Chung KJ and Bennett JE. Infections caused by *Malassezia* species. In: Medical Mycology. Publisher: Lea & Febiger, Philadelphia. London 1992; chapter 8: pp 170-182.
11. Benham RW. The cultural characteristics of *Pityrosporum ovale*-a lipophilic fungus. J Invest Dermatol 1939; 2: 187-203.
12. Midgley G. The lipophilic yeasts: state of the art and prospects. Med Mycol 2000; 38: 9-16.
13. Weiss R, Raabe, Mayser P. Yeasts of the genus *Malassezia*: taxonomic classification and significance in (veterinary and) clinical medicine. Mycoses 2000; 43: 69-72.
14. De Hoog GS, Guarro J, Gene J, Figueras MJ. Basidiomycetous yeasts. Genus: *Malassezia*. In: Atlas of Clinical Fungi. 2nd edition. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. 1998; pp 144-155.
15. Nazzaro Porro M, Passi S, Caprilli F, Nazzaro P, Morpurgo G. Growth requirements and lipid metabolism of *Pityrosporum orbiculare*. J Invest Dermatol 1976; 66: 178-82.
16. Leeming JP, Notman FH. Improved methods for the isolation and enumeration of *Malassezia furfur* from human skin. J Clin Microbiol 1987; 25: 2017-9.
17. Hammer KA, Riley TV. Precipitate production by some *Malassezia* species on Dixon's Agar. Med Mycol 2000; 38: 105-7.
18. Sternberg TH, Keddie FM. Immunofluorescence studies in tinea versicolor. Arch Dermatol 1961; 84: 999-1003.
19. Damert GJ, Kirpatrick CH, Sohnle PG. Comparison of antibody responses in chronic mucocutaneous candidiasis and tinea versicolor. Int Arch Allergy Appl Immunol 1980; 63: 97-104.
20. Faergemann J. Antibodies to *Pityrosporum orbiculare* in patients with tinea versicolor and controls of various ages. J Invest Dermatol 1983; 80: 133-5.
21. Midgley G, Hay RJ. Serological responses to *Pityrosporum (Malassezia)* in seborrhoeic dermatitis demonstrated by ELISA and western blotting. Bull Soc Fr Mycol Med 1988; 17: 267-76.
22. Johansson S, Faergemann J. Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *Pityrosporum orbiculare*. J Med Vet Mycol 1990; 28: 257-60.
23. Silva V, Fischman O, de Camargo ZP. Humoral immune response to *Malassezia furfur* in patients with pityriasis versicolor and seborrheic dermatitis. Mycopathologia 1997; 139: 79-85.
24. Faggi E, Pini G, Campisi E, Gargani G. Anti-*Malassezia furfur* antibodies in the population. Mycoses 1998; 41: 273-5.
25. Roberts SBO. *Pityrosporum orbiculare*. Incidence and distribution on clinically normal skin. Br J Dermatol 1969; 81: 264-9.
26. Faergemann J, Fredriksson T. Age incidence of *Pityrosporum orbiculare* on human skin. Acta Derm Venereal 1980; 60: 531-3.
27. Bergbrant IM, Faergemann J. Variations of *Pityrosporum orbiculare* in middle-aged and elderly individuals. Acta Derm Venereal 1988; 68: 537-40.
28. Silva V, Di Tilia C, Fischman O. Skin colonization by *Malassezia furfur* in healthy children up to 15 years old. Mycopathologia 1996; 132: 143-5.
29. Makimura K, Murayama SY, Yamaguchi H. Detection of a wide range of medically important fungi by the polymerase chain reaction. J Med Microbiol 1994; 40: 358-64.
30. Makimura K, Tamura Y, Kudo M, Uchida K, Saito H, Yamaguchi H. Species identification and strain

typing of *Malassezia* species stock strains and clinical isolates based on the DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Med Microbiol 2000; 49: 29-35.
31. Cotterill JA, Cunliff WJ, Williamson B, Bulusu L. Age and sex variation in skin surface lipid composition and sebum excretion rate. Br J dermatol 1972; 333-40.
32. Faergemann J, Ali R, Maibach HI. Quantitative variation distribution of *Pityrosporum orbiculare* in clinically normal skin. Acta Derm Venereal 1983; 63: 346-8.