

Identification of Different Malassezia Species Isolated from Skin of Healthy Dog Owners in Tabriz, Iran

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Abstract: Malassezia species are commensal organisms of human and animal skin that occasionally act as opportunistic pathogens. The lipid-dependent species are associated with human skin disorders whereas the non-lipid-dependent species (*Malassezia pachydermatis*) is considered as an opportunistic secondary pathogen affecting the canine skin surface and ear canal. The purpose of this study was to evaluate the possibility of canine malassezia organisms (Pachydermatis species) present on the body sites exposed to contact with the animal using the Culture Method; in another side to identify and isolate different species of Malassezia on the skin of studied individuals. In this study which performed in Tabriz, skin samples were obtained from 40 clinically healthy individuals who owned pet dogs. The Skin Brush Method was used for collecting skin samples. These samples were collected from four body sites including the chest, neck, palm and the interdigital surfaces of the fingers for the microscopic examination and culture. The frequency of malassezia infection was 30% among pet owners. The isolated species were *M. japonica* (31%), *M. globosa* (18.8%) and the species with unknown culture pattern (50%). The most common site among the studied sites was the chest (with 80% infection). Not isolating Pachydermatis species from the dog owners can show the low probability of infection transmission resulting from dogs and human beings relations, nevertheless if the malassezia infection exists, the possibility of the infection transmission should be considered.

Key words: Malassezia species, culture, isolation, dog owners, relations infection, Iran

INTRODUCTION

Malassezia organisms are lipophilic, non-mycelial, unipolar budding yeasts characterized by a thick cell wall (Simmons and Gueho, 1990; Yarrow and Ahearn, 1984). Yeasts of the genus Malassezia are known to be components of the microflora of human skin and that of many warm-blooded animals (Raabe *et al.*, 1998) but are also associated with a variety of diseases. Best known and most frequent is Pityriasis Versicolor (PV) (Mayer *et al.*, 1998), a chronic and recurrent skin disease occurring primarily in hot and humid climates.

Malassezia species have been recently reclassified on the basis of morphology, genomic composition and physiological characteristics of the yeasts (Cafarchia *et al.*, 2005). Currently, 10 species are included in the Malassezia genus: 9 lipid-dependent species (*M. dermatis*, *M. furfur*, *M. globosa*, *M. japonica*, *M. nana*, *M. obtusa*, *M. restricta*, *M. slooffiae* and *M. sympodialis*) and only 1 non-lipid-dependent species (*M. pachydermatis*) (Cafarchia *et al.*, 2005).

The 9 lipid-dependent species colonize the seborrheic part of the skin and they have been reported to be associated with pityriasis versicolor, seborrheic

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dermatitis, malassezia folliculitis and atopic dermatitis whereas *M. pachydermatis* is considered to be an opportunistic secondary pathogen growing on the skin surface and in the ear canal of pet carnivores (Guillot and Bond, 1999; Mason and Evans, 1991). Occasionally, *M. pachydermatis*, possibly of canine origin has been reported to cause nosocomial systemic infection in preterm newborns and immunocompromised human beings (Mickelsen *et al.*, 1988; Welbel *et al.*, 1994; Morris, 2005; Fan *et al.*, 2006; Guillot and Bond, 1999; Marcon and Powell, 1992; Chang *et al.*, 1998; Chryssanthou *et al.*, 2001). The purpose of the current study was to evaluate the possibility of canine malassezia organisms (*Pachydermatis* species) present on the body sites exposed to contact with the animal using the Culture Method in another side to identify and isolate different species of malassezia on the skin of studied individuals.

MATERIALS AND METHODS

Population and sampling: The current study was conducted during a period of January, 2010-2011 on 40 skin samples obtained by convenience sampling method from clinically healthy dog owners referring to 10 small animal clinics in Tabriz, a town in Northwest of Iran.

Skin Brush Method was used for sampling of the skin. Briefly, skin samples were collected from four sites of body including chest, neck, palm and interdigital surfaces of the fingers. All participants completed a questionnaire about their health status and other factors like age, sex, location, etc.

Culture and Isolation Methods: Collected brush samples were immediately inoculated onto Modified Dixon's Agar (MDA) as a selective media containing cycloheximide (0.05%) and chloramphenicol (0.05%) and incubated at 31°C for a period of 7 days. Direct observation of colonies was done by using light microscopy on smears that were prepared by methylene blue staining. All glass slides were observed with high magnification immersion oil microscopy technique. Yeast size having budding cells, presence of other microbiological agents and the number of budding per yeast were the major criteria for perfect diagnosis of the cultured yeasts. After confirmation of the yeast colonies by microscopic examination, they are inoculated onto MDA medium again for further purification and pure isolated yeasts went under differentiation cultures using different types and dilutions of Tween as a sole lipid source. Briefly, MDA medium and Sabouraud Glucose Agar (SGA), containing cycloheximide (0.05%) and chloramphenicol (0.05%)

supplied with tweens 20, 40, 60 and 80 (0.1-10%) were used for further identification of yeast species. The tweens containing cultured media were incubated at 31°C for 7 days and assimilation of the tweens by yeast was considered. Morphological, physiological and metabolic characteristics for recognition of species performed based on method (Khosravi *et al.*, 2008).

Physiologic characteristics

Catalase reaction: The presence of catalase was determined by using a drop of hydrogen peroxide (3% solution) and production of gas bubbles was considered as a positive reaction. *M. restricta* is the only Malassezia species that exhibit no catalase activity. *M. pachydermatis* isolates have variable reaction (Khosravi *et al.*, 2008).

Demonstration of *M. pachydermatis*: As *M. pachydermatis* is the only Malassezia species that is not obligatory lipid dependent, the yeasts isolated on mDixon agar were smeared with a sterile swab on Sabouraud Glucose Agar (SGA) plus 0.05% cycloheximide and chloramphenicol (0.05%) devoid of lipids. Incubation was performed at 31°C for 1 week (Khosravi *et al.*, 2008).

Tween assimilation test: According to the method reported by Guillot ability to utilize different Tween compounds as a unique lipid supplement by Malassezia species was evaluated. Briefly, yeast suspension ($2 \times 3 \times 10^5$ cfu mL⁻¹) was made in 1 mL sterilized distilled water and poured into plate containing SGA with 0.05% cycloheximide and chloramphenicol (0.05%) that cooled to about 50°C.

The inoculum was then spread evenly. After solidification of each plate, four holes were made by means of a 3 mm diameter punch and filled with 5 µL of Tween 20, 40, 60 and 80, respectively. The plates were incubated for 1 week at 31°C. Utilization of Tweens was assessed by the degree of growth and/or reaction (precipitation) of the lipophilic yeasts around the wells (Khosravi *et al.*, 2008).

Splitting of esculin: To improve the differential diagnosis of different species of Malassezia, the α-glucosidase activity was assessed using esculin agar tube. A loop of fresh (2-3 days old) yeasts was deeply inoculated into the agar and incubated at 31°C for 5 days. The splitting of esculin into esculetin and glucose is revealed by darkening of the medium with liberation of soluble ferric salt incorporated in the medium. *Listeria monocytogenes* and *Streptococcus agalactiae* served as positive and negative control, respectively.

Significant brown staining of more than a third of the medium is considered *M. sympodialis* and *M. obtusa*. *M. furfur* causes weak staining. *M. pachydermatis* has a variable reaction. The other species are negative (Khosravi *et al.*, 2008).

RESULTS AND DISCUSSION

Of 40 individuals that owned pet dogs, 60% were male. Based on the findings of this study, the frequency of Malassezia infection was 30% among pet owners from which 66.7% were males and 33.3% females. Among these individuals, 9 people (75%) were positive in only one site and 3 ones (25%) in two sites.

The physiological and biochemical characteristics of different Malassezia species been shown in Table 1 (Khosravi *et al.*, 2009). Using the integrative identification system, of 16 positive culture cases, 8 cases (50%) were recognized as unknown or atypic Malassezia species, 5 cases (31.3%) as *M. japonica* and finally 3 cases (18.8%) as *M. globosa*. The other Malassezia species and also *M. pachydermatis* were not isolated from the referring individual's skin. The frequency of isolated species has been reported based on the studied anatomical sites and sex factor in Table 2. Among the studied sites, the chest was the most frequent site of the Malassezia infection (80%) and next sites were the neck (13.3%) and palm (6.7%) sites, respectively. Also in Table 3, the infection frequency in both sexes and different anatomical sites are given.

Yeasts of the genus Malassezia are components of the normal microflora of human skin and that of many warm-blooded animals. These organisms can induce pityriasis versicolor and have an important role as an aggravating factor in several skin diseases such as atopic dermatitis and seborrheic dermatitis, especially in atopic dermatitis they act as an exacerbating allergic factor. Thus, the accurate identification of Malassezia species is of high significance for the recognition and determination of malassezia yeast pathogenesis in various cutaneous diseases. The purpose of the current study was to

determine the possibility of *M. pachydermatis* species present on the body sites exposed to the animal contact. Moreover, the isolation and identification of different Malassezia species on the studied individual's skin were conducted because of using the Culture Method. In this study, the frequency of Malassezia infection among pet owners was 30% and the most frequent isolated species were *M. japonica* and *M. globosa*, respectively. The most common site was chest followed by neck and palm. Different frequencies and species were reported in other studies. In one study conducted by Zomorodian *et al.* (2008) in Tehran, the frequency of infection was reported to be 87% and the most common isolated species from the clinically healthy individual's skin were *M. globosa* and *M. furfur* which has less congruence with the current study. It seems that the differences observed in this study and the study done in Tehran can be resulted from the individual differences (sanitary, sebaceous glands density on the skin, etc.) geographical and climatic ones.

Regarding that this yeast needs high temperatures and quite high humidity, Tehran is susceptible but in Tabriz which is a mountainous city with approximately low temperature and dry climate, probably the optimum conditions for the growth and propagation of Malassezia organisms are not so suitable.

In a research done by Lee *et al.* (2006) in Korea using the Culture Method, the most cases of positive culture was on the chest which is in agree with this study. The frequency of Malassezia infection was 66% according to this study and the most common isolated species were *M. restricta* and *M. globosa*, respectively the obtained outcome is somewhat parallel to the current study. Moreover in this study, beside these two species the *M. sympodialis*, *M. slooffiae* and *M. furfur* species were also isolated which have not been isolated from human skin in the current study (Lee *et al.*, 2006).

In another study by Oh *et al.* (2009) in Korea, the rate of Malassezia infection based on the Culture Method was 63.6% and the most common identified species using PCR technique were *M. globosa*, *M. restricta*, *M. sympodialis*, *M. furfur*, *M. dermatis* and *M. slooffiae*,

Table 1: Biochemical and physiological characteristics in nine species of Malassezia

Species	Growth on SDA	Growth on mDixon at 32°C	Utilization of				Splitting of esculin	Catalase reaction
			10% TW 20	0.5% TW 40	0.5% TW 60	0.1% TW 80		
<i>M. pachydermatis</i>	+	+	+	+	+	+	V	V
<i>M. sympodialis</i>	-	+	-	+	+	+	+	+
<i>M. globosa</i>	-	+	-	-	-	-	-	+
<i>M. dermatis</i>	-	+	+	+	+	+	-	+
<i>M. furfur</i>	-	+	+	+	+	+	-	+
<i>M. slooffiae</i>	-	+	± or +	+	+	-	-	+
<i>M. obtusa</i>	-	+	-	-	-	-	+	+
<i>M. restricta</i>	-	+	-	-	-	-	-	-
<i>M. japonica</i>	-	+	-	±	+	-	+	+

mDixon: Modified Dixon agar; Tw: Tween; +: Positive; -: Negative; ±: Weakly positive; V: Variable reaction

Table 2: Frequency of Malassezia isolates based on anatomical sites and sex

Malassezia isolates	Anatomical site			Sex	
	Chest (%)	Neck (%)	Palm (%)	Male	Female
<i>M. japonica</i>	4 (23.5)	0	1 (5.9)	3 (21.4)	1 (7.1)
<i>M. globosa</i>	3 (17.6)	1 (5.9)	0	0	3 (21.4)
<i>M. unknown</i> sp.	6 (35.3)	2 (11.8)	0	6 (42.9)	1 (7.1)
Total	13 (76.4)	3 (17.7)	1 (5.9)	9 (64.3)	5 (35.6)

Table 3: Frequency of Malassezia infection based on anatomical sites and sex

Sex	Anatomical site		
	Chest (%)	Neck (%)	Palm (%)
Male	8 (53.3)	1 (6.7)	1 (6.7)
Female	4 (26.7)	1 (6.7)	0
Total	12 (80)	2 (13.4)	1 (6.7)

respectively which is different from the results of the current study. But in another study in Korea conducted by Kwon *et al.* (1999) using the Culture Method, the chest was the most common site of infection which is in accordance with the results of the current study.

The most frequent isolated species in this study was *M. restricta* species which is different with the results of the current research. In a study by Leeming *et al.* (1989) using the same technique, the most frequent sites were the chest, the upper part of back and forehead sites which partially supports the results of this study. Based on results of this study and other reports it seems that chest can be considered as most frequent site of infection but isolating the different species can show the growth of the different Malassezia species under various sanitary, climatic and racial circumstances.

In another study by Faergemann and Maibach (1983) by Culture Method, the scalp and forehead sites were the most frequent sites of infection, respectively. Because in the current study, the culture sample has not been obtained from the mentioned areas, it is impossible to compare the results of two cited studies.

In another study conducted by Sugita *et al.* (2001) in Japan, the frequency of infection has been reported 78% using molecular technique, nested-PCR and *M. restricta*, *M. sympodialis*, *M. globosa* and *M. furfur* species were respectively, the most common identified species which as it appears is different with the findings of the current study.

It seems that the reason why the before-mentioned findings are different from the results of the current study is the racial, sanitary and climatic differences plus the amount of obtained samples besides using advanced diagnostic methods such as PCR; regarding few number presence of Malassezia yeasts on the healthy skin, the possibility of success in Culture Method is low but in PCR Method due to its high sensitivity and its nature which is based on molecular diagnosis, the probability of infection of the studied individuals is higher.

It seems that the observed differences of the infecting species and rate of the infection on different anatomical sites of the body are related to the different amount of skin sebum on the different sites of the body. It is assumed that the chest is susceptible to the higher infection comparing with the other parts of the body due to the high density of sebaceous glands in this part and the high amount of sebum secretion. But not isolating the *Pachydermatis* species from the animal owners can show the low probability of the infection transmission resulting from human relation with dogs.

CONCLUSION

This study shows that the infection by *Pachydermatis* species in human beings is very limited to individuals and is mostly noticeable in immunocompromised people and preterm newborns (Welbel *et al.*, 1994; Morris, 2005; Fan *et al.*, 2006; Guillot and Bond, 1999; Marcon and Powell, 1992; Chang *et al.*, 1998; Chryssanthou *et al.*, 2001). Therefore, it seems that considering the unsuccessful culture of the mentioned organism, the probability of accidental infection in animal owners is very low and can be overlooked.

Regarding the results of the current study, it is apparent that some cultured species in the dog owners using the common methods of the culture technique have not been identified that results both from the probability of the infection with two Malassezia species which can not be identified using the current Culture Method (*M. nana* and *M. yamatoensis* species) and from the common presence of Atypic species which show different characteristics unlike the typical pattern of the Culture Method (Kaneko *et al.*, 2007) and also results from the presence of the new probably unknown species because of mutation or such issues. So, it is required to do more extensive researches using more advanced methods like PCR. It must be approved that using the Culture Method due to high specificity can provide the diagnostic laboratories with the ability of identifying different species and this method can be considered in the presence of clinical signs indicating Malassezia infection because this method unlike more advanced ones such as PCR does not need advanced units and lots of expenses. Moreover, the Culture Method unlike PCR is available in majority of diagnostic centers but it should be said that the sensitivity of the Culture Method is lower comparing with the golden standard (PCR) (Morris, 2005) but higher comparing to Cytolog Method. It is to be noticed that the Cytology Method comparing with the Culture Method possesses a good relative specificity but very low relative sensitivity (Cafarchia *et al.*, 2005). Therefore, it is recommended that for ongoing researches both the

Culture and PCR Methods are used simultaneously so that the advantages and disadvantages of both techniques in aspect of the cost, time-consumption, need for trained personnel, sensitivity and specificity could be evaluated more precisely.

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