

Original Research Article

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## ***Malassezia* Species Associated With Dermatitis in Dogs and Their Antifungal Susceptibility**

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### **A B S T R A C T**

The present study was taken with the objective of isolation, characterization, molecular detection and antifungal sensitivity of *Malassezia* species from dermatitis cases from dogs in and around Pondicherry state. A total of 100 skin swabs were collected from the dogs showing dermatological problems suggestive of *Malassezia*. Out of 100 swabs, 41 *Malassezia* isolates were successfully isolated and had good growth on Sabouraud's Dextrose Agar (SDA) during the primary isolation from the skin swabs. Biochemical tests for catalase,  $\beta$ -glucosidase activities and the capability to grow with three water soluble lipid supplements, namely Tween 20, Tween 80 and Cremophor EL concluded that the *M. pachydermatis* was the sole species isolated from the cases of canine dermatitis in Pondicherry state. Cytological examination revealed that direct skin swab smear was more sensitive than adhesive tape technique and impression smear. The frequency of isolation of *M. pachydermatis* was higher in neck region (8) followed by other regions in canine. Out of 100 skin swabs screened using an *M. pachydermatis* species specific primers, 61 were identified positive. The study showed a higher sensitivity of PCR (61%) in detecting *Malassezia* dermatitis over culture (41%). Based on *invitro* antifungal susceptibility studies it can be concluded that Ketoconazole, Itraconazole, Fluconazole and Amphotericin B can be advocated as the drug of choice to treat *Malassezia* dermatitis in this geographical location.

#### **Keywords**

*Malassezia pachydermatis*, Sabouraud's dextrose agar, Modified Dixon's agar, Polymerase chain reaction, Antifungal Susceptibility testing

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### **Introduction**

*Malassezia* has been classified as dimorphic fungi since it has been found to exist in both yeast and mycelial phases.

The yeast form was named as *Pityrosporum* and the mycelial form as *Malassezia*. However, in 1986 they were recognized to be two forms of the same organism and were thus

collectively named *Malassezia* (Cannon, 1986). *Malassezia* considered as a normal cutaneous inhabitant of skin in humans, animals and birds. These lipophilic fungi colonize in the stratum corneum layer of skin, which is rich in lipids. These Dimorphic lipophilic fungi sometimes act as opportunistic pathogens and cause the dermatological infections such as seborrhoeic dermatitis and otitis in domestic animals.

The *Malassezia* yeast cells are round, oval or cylindrical and may vary in size from 1 to 8µm diameter (Keddie, 1966). They reproduce asexually by budding from a broad base (Chen and Hill, 2005). The cell wall of *Malassezia* is very thick (0.12µm) and multilayered, consisting mainly of sugars (70%), lipids (15-20%) and proteins (10%) (Ashbee and Evans, 2002). All *Malassezia* species except *Malassezia pachydermatis* requires an exogenous source of long chain fatty acids like C14 or C16 fatty acids for their growth.

The genus *Malassezia* belongs to the Phylum Basidiomycota, comprises of 14 species, which have been identified based on their morphology, biochemical features and molecular analysis. Initially, seven species (*M. furfur*, *M. obtusa*, *M. globosa*, *M. slooffiae*, *M. sympodialis*, *M. pachydermatis* and *M. restricta*) were described (Gueho *et al.*, 1996). Later, seven new *Malassezia* spp. (*M. dermatis*, *M. equina*, *M. japonica*, *M. nana*, *M. yamatoensis*, *M. caprae* and *M. cunicoli*) were identified using biochemical, morphological, biological and molecular analysis (Sugita *et al.*, 2004; Hirai *et al.*, 2004; Cabanes *et al.*, 2007).

*M. pachydermatis* produces proteolytic enzymes that can damage the epithelium leading to hyperplasia with enlargement of the ceruminous glands (Nicklas and Mumme, 1979). *Malassezia* produces keratinase and other enzymes capable of digesting the keratin protein complex, allowing the organism to burrow deeper into the stratum corneum in the host and elicit an inflammatory reaction (Guillot, 1995).

The lesions are usually first seen on the abdominal skin, but may spread to the entire abdomen, the axilla and the inguinal region (Larsson *et al.*, 1988). In diseased ears, however, *M. pachydermatis* can be readily found alone or associated with bacteria,

mainly *Staphylococcus* spp. *M. pachydermatis* can also be isolated from the ears of clinically healthy animals (Mansfield *et al.*, 1990).

Histopathologically, the lesions are characterized by inflammation, perivascular infiltration, epidermal and follicular hyperkeratosis with many budding yeast cells in the stratum corneum (Mason and Evans, 1991). The organism is not restricted to dogs alone, as it has been isolated from several other mammalian species and birds (Scott, 1992). Zoonotic transfer has been documented from dogs to immunocompromised patients by health care workers who own dogs (Chang *et al.*, 1998).

Dermatitis due to *Malassezia* species in canines is recognized with increasing frequency in veterinary practice in Pondicherry state (Southern India). Since no systematic investigations were carried out with regards to the occurrence and treatment of *Malassezia* infections in this region, the present study was aimed with the Isolation, characterization followed by PCR-based detection and Antifungal sensitivity of *Malassezia* species from dermatitis dogs cases in and around Pondicherry state.

## **Materials and Methods**

### **Sample collection and Processing**

A total of 100 skin swabs were sampled from the dog cases came to the Teaching Veterinary Clinical Complex, RIVER, Pondicherry (33 nos) and Animal husbandry department, Pondicherry (67 nos) showing dermatological problems suggestive of *Malassezia* dermatitis like erythema, hyperpigmentation, greasy exudates, scaling etc (Supplementary Figure 1).

Sterile cotton swabs moistened with sterile distilled water were used to collect the

specimen. The cotton swab was rolled and rubbed firmly against the entire skin area for 10 seconds from the dogs showing dermatological problems suggestive of *Malassezia* dermatitis. The swab was then transferred onto the sterile test tube and transported to laboratory within 2 to 4 hours of the collection.

### **Cytological studies**

#### **Impression smear examination**

The impression Smears were made in a clean glass slide directly from the surface of the lesion. Then the heat fixed impression smears were stained with Methylene blue stain to detect the presence of *Malassezia* yeasts under oil immersion objective. More than one yeast cell per field is considered positive for *Malassezia* dermatitis (Nardoni *et al.*, 2008).

#### **Adhesive tape examination (Scotch test)**

Here the impression is made with the adhesive surface of the cellophane tape placed onto the affected skin surface firmly for 2 - 3 sec and then stuck onto a clean microscopic slide containing 2-3 drops of lactophenol cotton blue (LCB) mount. The tape strip stained with LCB mount for 2 minutes was examined under high power objective microscope to detect the presence of *Malassezia* yeasts.

#### **Skin swab smear**

The smears are made by rolling / rubbing the wet cotton swab firmly against the entire skin lesions area for 10 seconds and then rolled / rubbed over the microscopic glass slide. Then the heat fixed impression smears were stained with Methylene blue stain to detect the presence of *Malassezia* yeasts under oil immersion objective. More than one yeast cell per field is considered positive for *Malassezia* dermatitis (Nardoni *et al.*, 2008).

### **Isolation and identification**

The skin swabs were plated on Sabouraud's dextrose agar (SDA) and Modified Dixon's agar (MDA) and incubated at 37°C for 7 days for the isolation of *Malassezia* yeasts. The colonies showing cultural characters of *Malassezia* were examined microscopically for the typical morphology and were further subjected for a) Catalase activity, b)  $\beta$  glucosidase test, c) Tween and Cremophor EL utilization biochemical tests to identify them upto species level (Cafarchia *et al.*, 2011).

#### **Catalase activity**

A loopfull of test culture on SDA agar was mixed with 2-3 drops of 3 per cent H<sub>2</sub>O<sub>2</sub> on a clean glass slide and examined for the release of nascent oxygen in the form of gas bubbles. A positive reaction was indicated by the effervescence of oxygen within 1-2 min. (Gueho *et al.*, 1996).

#### **$\beta$ glucosidase test**

$\beta$  glucosidase test was done by inoculating a loop full of fresh culture into esculin iron agar medium and incubated at 37°C for 5 days. A positive reaction was indicated by the blackening of the medium which reveals that *Malassezia* organism possess a  $\beta$  glucosidase that is able to hydrolyse the glucosidic bond of esculin, thus liberating glucose and esculin. The phenol moiety reacts with the iron to give a black colour (Kaneko *et al.*, 2006).

#### **Tween and Cremophor EL utilization**

Two loops of a 4 to 5 day old *Malassezia* culture were suspended in 3.0ml of sterile demineralised water. This inoculum was added to molten SDA maintained at 50°C, and the mixture was poured immediately in a 9 cm petri dish. After complete solidification, wells were made with a 2 mm punch, devoted to test

the growth using Tween 20, Tween 80 and Cremaphor EL. The wells are filled with approximately 15µl of each product and petri dishes are incubated for 7 to 10 days at 37°C in a moist environment, and turned upside down on the second day to delay their dehydration. A positive reaction was indicated by presence of dense growth around the wells in a disk like pattern.

### **Antifungal susceptibility test**

Antifungal susceptibility test was carried out as per the standard disc diffusion method described by Bauer *et al.*, (1966). Sabouraud's dextrose agar (pH 5.6 ± 0.2) was employed for antifungal susceptibility test. Three to five pure individual colonies were inoculated into 3ml of 0.04% Tween 80 and incubated at 37°C for 2hr. Then the lawn culture was made with the swab from the incubated inoculums onto the SDA plates. After lawn culture was made the SDA plates were placed in the refrigerator for 2hr for better absorption of the culture. Following antifungal discs with the mentioned concentrations were applied over the plated SDA. Itraconazole (It) 10µg, Ketoconazole (Kt) 10µg, Nystatin (Ns) 100 I.U, Fluconazole (Fu) 10µg, Amphotericin B (Ap) 100 I.U, Clotrimazole (Cc) 10µg, Miconazole (Mic) 30µg. The Antifungal impregnated plates were incubated in the inverted position, at 37°C for 48 hr. The interpretation of zone diameter was carried out according to the standards laid down by Clinical Laboratory Standards Institute (CLSI), formerly known as National Committee for Clinical Laboratory Standards (NCCLS). The diameter of zone of inhibition was translated into sensitive or resistance.

### **Polymerase Chain Reaction**

Boiling-lysis method of extraction of DNA from the clinical sample and culture recommended by Zhang *et al.*, (2004) with

slight modifications was followed. The clinical sample / culture was boiled in a water bath at 96°C for 10 min followed by snap chilling for 5 mins and centrifugation at 16,000g for 5 min. The supernatant was used as a template DNA in a PCR reaction.

The PCR amplification was carried out with the species specific primer pair M.pa<sub>for</sub> & M.pa<sub>rev</sub> with the Initial denaturation at 94°C for 3 min, followed by 30 cycles at final denaturation at 94°C for 30s, annealing at 62°C for 1 min, extension at 72°C for 40s and a final extension at 72°C for 10 min. Following PCR, the amplified product was analyzed by submergane gel electrophoresis

## **Results and Discussion**

### **Isolation and identification**

Samples collected from 100 dogs with symptoms suggestive of *Malassezia* dermatitis were incubated at 37°C for 7 days on SDA and 15 days on MDA. Growth of *Malassezia* was observed from 4 to 7 days on SDA whereas on MDA the growth was observed from 9 to 15 days. Based on this result, it could be concluded that SDA is a preferable medium for the isolation of *Malassezia pachydermatis* compared to MDA although many workers have suggested the superiority of Modified Dixon's agar for the isolation of lipid dependent species of *Malassezia* from canine dermatitis (Nardoni *et al.*, 2006, Galuppi *et al.*, 2010 and Cafarchia *et al.*, 2011).

Out of 100 samples collected, 41 isolates of *Malassezia* spp. were isolated successfully. The colonies of *Malassezia* spp. were macroscopically visible over 3-5 days when incubated at a temperature of 37°C whereas; the growth was weak when incubated at room temperature (25<sup>0</sup> C). The colonies were raised or high convex and smooth with cream colour

initially (Figure 1) and later became dry, wrinkled and orange to brown in colour (No shown). On microscopic examination of the isolated colonies using Grams staining procedure; the organisms appeared dark blue colored with unique peanut or footprint shaped (Figure 2).

### **Biochemical tests for *Malassezia* species**

The identification of *Malassezia* yeasts is done by using specific biochemical tests like catalase test,  $\beta$  glucosidase activities and the capability to grow with three water soluble lipid supplements, namely Tween 20, Tween 80 and Cremophor EL. In this research, all the 41 *Malassezia* yeast isolates had a very slow or weak activity for catalase. These results are similar to those of Galuppi *et al.*, (2010) who have reported very weak catalase activity in 23 strains of *M. pachydermatis*. Similarly, all the 41 *Malassezia* yeast isolates were also positive for  $\beta$  glucosidase activity. 41 *Malassezia* isolates utilized Tween 20, Tween 80 and Cremophor EL by showing good growth around them (Figure 3). These results were similar to Galuppi *et al.*, (2010) who recorded good growth around the wells of Tween 20, Tween 80 and Cremophor EL. Based on the above tests in isolates, the study concludes that *M. pachydermatis* was the sole species isolated from the cases of canine dermatitis. In this work, the lack of recovery of lipid dependent species could be ascribed to the strongly modified skin habitat due to atopy. These alterations could have determined an overgrowth of *M. pachydermatis* with the consequent inhibition of lipid dependent species, less adapted to the cutaneous microhabitat of dogs.

### **Polymerase chain reaction**

Out of 100 canine dermatitis clinical samples (skin swabs from the lesions) screened using an *M. pachydermatis* species specific primers

(Table 1) derived from the internal transcribed spacer region of the rRNA gene, 61 were found positive with the expected product size of 220 bp (Figure 4). Culture evidence indicated that only 41% of the cases were positive whereas 61% of cases were positive for *Malassezia* infection by PCR assay. Chi square analysis also revealed significant differences ( $P < 0.5$ ) between isolation and PCR assay in the diagnosis of *Malassezia pachydermatis*.

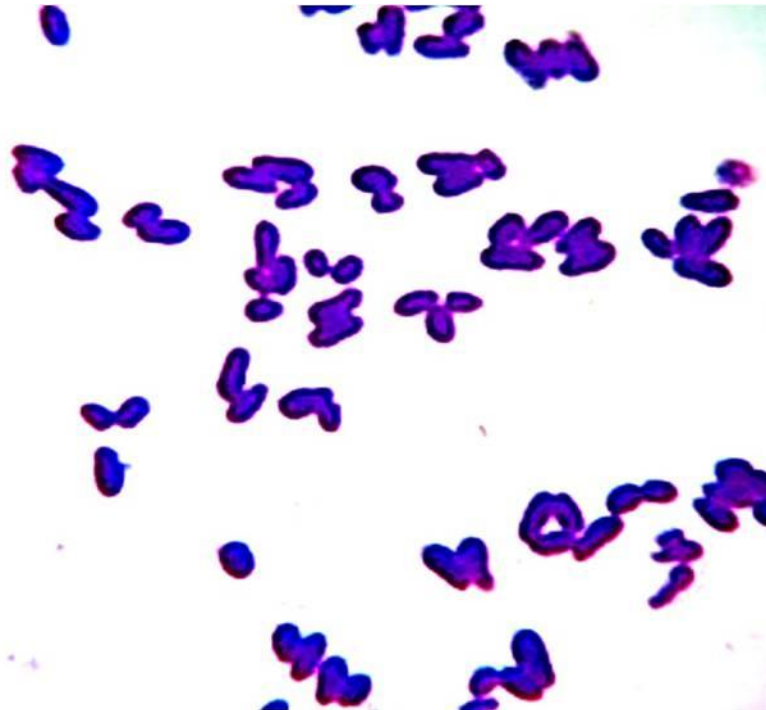
Among the 100 infected animals included in this study, the highest occurrence was noticed in dogs of one to three years of age (56%) followed by six months to one year of age (19%), three to six years of age (16%), zero to six months of age (6%) and above six years of age (3%). Age wise occurrence of *Malassezia* infections is depicted in Table 2. This is in concurrence with the earlier reports of Girao *et al.*, (2006) who opined that majority of dogs with *M. pachydermatis* were aged between one and three years of age.

The study showed that *Malassezia* dermatitis was markedly prominent in Non- descriptive dogs (55%) followed by Labrador Retriever and Spitz (13%), German Shepherd (11%) Chinese Pug (4%), Dalmatian (2%), Doberman and Great Dane (1%). The detailed breed wise occurrence of *Malassezia* dermatitis is presented in Table 3. Certain breeds like American cocker spaniel, Springer spaniel, Basset Hound, Daschund, English setter, West Highland terrier, Silky terrier, German Shepherd, Poodles, Chihuahua and Collie are reported to be at increased risk (Plant *et al.*, 1992, Bond *et al.*, 1996, Mauldin *et al.*, 1997, Charach 1997 and Muller *et al.*, 2001). In Puducherry region, most of the middle and low income groups of people were rearing Non descriptive breeds of dogs due to their economic status. This might have influenced the highest percentage of incidence of disease among Non-descriptive dogs.

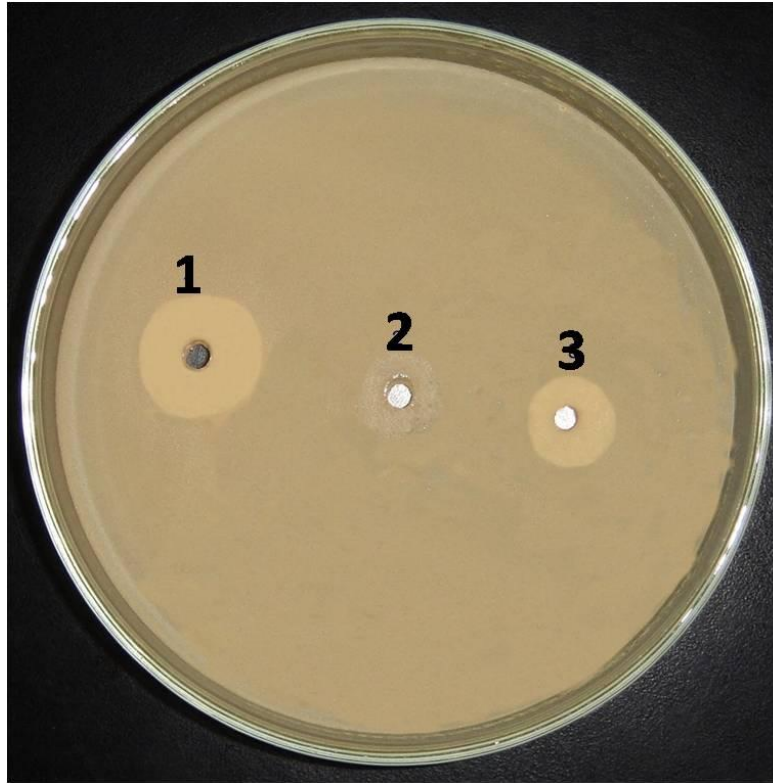
**Fig.1** Growth of *M. pachydermatis* was observed on an average of 4 to 7 days on SDA which were incubated at 37°C. The colonies were raised, high convex, smooth with cream colour initially and later (not shown) became dry, wrinkled and orange to brown in colour



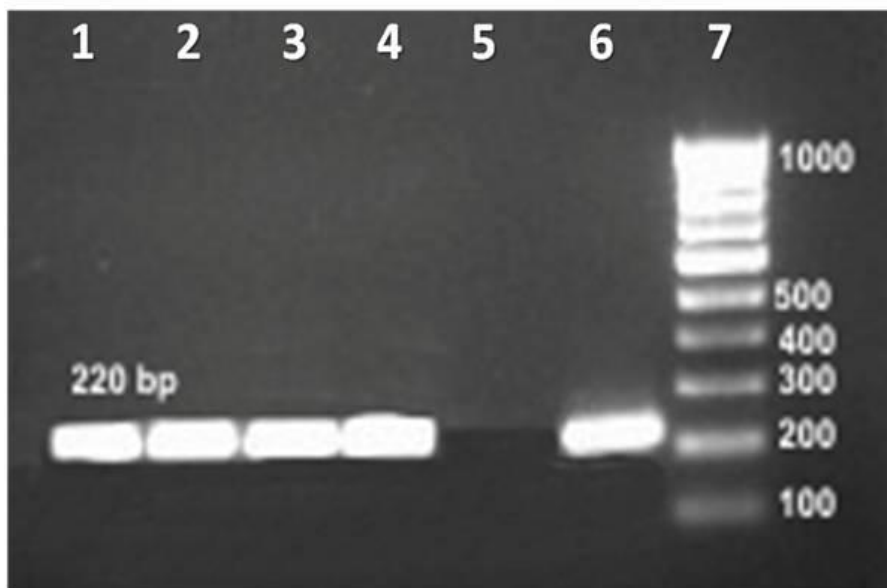
**Fig.2** Skin swab cultured isolate showing the microscopic morphology (1000X magnification). Peanut or footprint shaped budding yeast cells of *M. pachydermati* demonstrated by Methylene blue staining technique



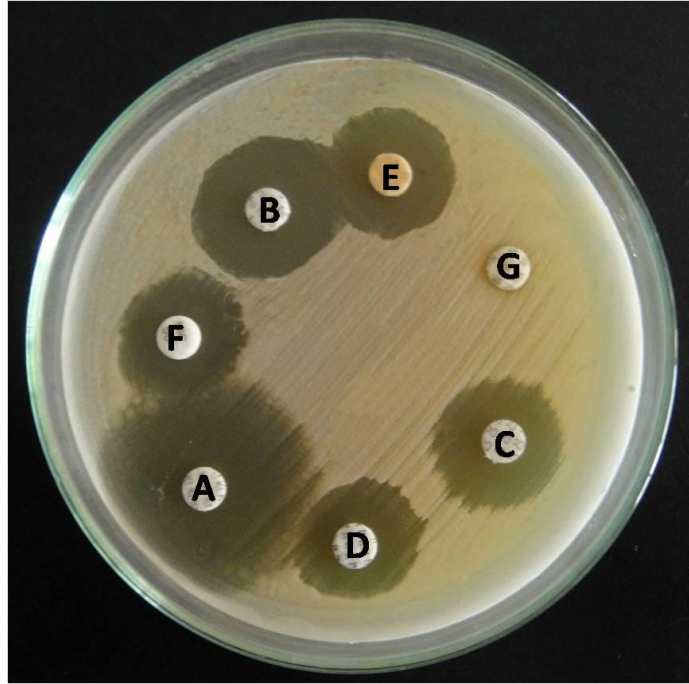
**Fig.3** Utilization of Tween 20, Tween 80 and Cremophor EL by *Malassezia* isolate; showing good growth around them. 1. Tween 80, 2. Cremaphor EL, 3. Tween 20



**Fig.4** Screening of Clinical swabs / isolates for *Malassezia pachydermatis* by PCR amplification using *M.pa*<sub>for</sub> & *M.pa*<sub>rev</sub> primer pair; Lane 1 & 2- Clinical samples Lane 3 & 4 – isolates, Lane 5 – Negative control, Lane 6 – Positive control & Lane 7 – DNA Marker



**Fig.5** Antifungal susceptibility test carried out with seven antifungal agents against one of the clinical isolate. The isolate shows that it is sensitive to Amphotericin B (A), Ketoconazole (B), Itraconazole (C), Fluconazole (D), Miconazole (E) and Clotrimazole (F) but resistant to Nystatin (G)



**Supplementary Fig.1** The dog cases came to the Teaching Veterinary Clinical Complex, RIVER, Pondicherry showing dermatological problems such as A) Scaling, B) Otitis externa, C) Hyperpigmentation and D) Erythema, etc suggestive of *Malassezia* dermatitis





**Table.1** Oligonucleotide primers used for amplification for *Malassezia pachydermatis* gene

Target	Primers	Sequence (5'→3' direction)	Amplicon size (bp)
<i>Malassezia pachydermatis</i>	M.pa <sub>for</sub>	CTGCCATACGGATGCGCAAG	220
	M.pa <sub>rev</sub>	TTCGCTGCGTTCTTCATCGA	

**Table.2** Age wise occurrence of *Malassezia* dermatitis in dogs

AGE	No. of Infected Animals
0 to 6 months	6
6 months to 1year	19
1 to 3 years	56
3 to 6 years	16
>6 years	3
<b>Total</b>	<b>100</b>

**Table.3** Breed wise occurrence of *Malassezia* dermatitis in dogs

Breed	No. of Infected Animals
Dalmatian	2
Doberman Pinsher	1
German Shepherd	11
Great Dane	1
Labrador Retriever	13
ND	55
Pug	4
Spitz	13
<b>Total</b>	<b>100</b>

**Table.4** Comparison of the Cytological Examinations

Cytological Examination	Cases positive (%)
Impression smear	26
Adhesive tape	34
Skin swab smear	40

**Table.5** Frequency of *M. pachydermatis* isolation from different locations of dogs

Location in dogs	No. of cultures obtained
Dorsal trunk	6
Ear pinnae	4
Fore legs	3
Generalized dermatitis	4
Hind legs	4
Interdigital area	1
Neck	8
Perenial region	1
Prescrotal region	4
Ventral trunk	6
<b>Total</b>	<b>41</b>

**Table.6** Antifungal susceptibility of *Malassezia pachydermatis* isolated from Canine Dermatitis

Antifungal drugs	Sensitive	Resistant
Miconazole	25	16
Ketoconazole	41	0
Itraconazole	41	0
Fluconazole	41	0
Clotrimazole	15	26
Nystatin	0	41
Amphotericin B	41	0

Based on sex wise occurrence, highest incidence was observed in Male (69 nos) over female (31 nos) for *Malassezia* dermatitis. The chi-square analysis revealed that there is a significant difference between male and female dog's in the occurrence of *Malassezia* dermatitis ( $P < 0.5$ ). In contrary to this study no significant sex predilection for *Malassezia* infection was detected by Bond *et al.*, (1996) and Nardoni *et al.*, (2004).

The frequency of isolation was higher in neck region (8 nos) followed by dorsal and ventral trunk (6 nos), ear pinnae, hind legs, prescrotal region and in cases of generalized dermatitis (4 nos), fore legs (3 nos), perenial region and interdigital area (1 no). The results of the present findings corroborates with the

observations of Yurayart *et al.*, (2010) who reported that highest number of isolates were obtained from the neck region followed by ear canal, interdigital area and groin.

#### **Cytological examination**

In impression smear examination, on an average 3-4 peanut or footprint shaped budding yeast cells per 1000X oil immersion field could be detected by Methylene blue staining. In adhesive tape smear examination, on an average 8-10 cells per field could be detected. Adhesive tape smear detected budding yeast cells more frequently when compared with direct impression smear examination. In skin swab smear examination, on an average 5-8 cells per field was detected.

The results obtained by these three methods are depicted in Table 4. In the present study 26% cases were positive by impression smear, 34% cases were positive by adhesive tape and 40% cases were positive by skin swab smear. This study indicated that the impression smear, adhesive tape and the dry swab sampling techniques, successfully detected the yeast *Malassezia* on the skin of dogs. However, when the results of the impression smear, tape and swab techniques were compared; it was found that the swab technique detected the yeast on significantly more dogs than the impression smear examination and adhesive tape. The chi-square test analysis reveals that there is a significant difference between impression smear examination versus skin swab smear and adhesive tape techniques ( $P < 0.0353$ ). The chi-square analysis also revealed that there is no significant difference between skin swab smear and adhesive tape techniques ( $P > 0.5$ ). Omodo *et al.*, (2003) reported that a swab is likely to be more practical than the adhesive tape when collecting material from the skin surface of a dog with a heavy greasy exudate as may be found in severe cases of seborrhoea. The exudate may prevent the tape from sticking to the glass and may also make it difficult to distinguish colonies of *M. pachydermatis* growing under the adhesive tape on culture. On the basis of the empirical evaluation of this study, skin swabs were considered to be the most reliable specimen because they may be used for both cytological examination and culture and are easy to use.

### **Antifungal susceptibility test**

The antifungal susceptibility test was carried out with seven antifungal agents against 41 isolates of *M. pachydermatis* as per the standard disc diffusion method. All the isolates were susceptible to Amphotericin B, Ketoconazole, Itraconazole and Fluconazole followed by 25 isolates were susceptible to

Miconazole and 15 isolates were susceptible to Clotrimazole (Figure 5). A cent percent sensitivity recorded in this study indicates that any of the above drugs can be suggested for the treatment of *M. pachydermatis* dermatitis in canines. Moreover, all the 41 isolates were resistant to Nystatin followed by 26 isolates resistant to Clotrimazole and 16 isolates resistant to Miconazole. The study reveals that Nystatin should not be recommended for the treatment of *M. pachydermatis* dermatitis in canines. Variations in the antifungal sensitivity were observed only with Miconazole and Clotrimazole. Details of the antifungal susceptibility test of *M. pachydermatis* to seven antifungal drugs are presented in Table 5.

Clinical resistance to antifungal drugs by *Malassezia* species has only rarely been reported in veterinary or human medicine, and while the majority of studies have shown little evidence for in vitro antifungal resistance, multiple reports have demonstrated occasional very high anti-fungal MICs in individual *Malassezia* species and strains (Robson *et al.*, 2010). Furthermore, resistance by *M. pachydermatis* has been shown to develop in vitro with multiple passage at near MIC concentrations of antifungals, suggesting that the cellular machinery exists in this species for development of possible clinically relevant resistance. More resistant species reported are *M. pachydermatis* (Nakamura *et al.*, 2000), *M. furfur* (Duarte *et al.*, 2006), *M. globosa* and *M. restricta* (Rincon *et al.*, 2006). Whereas *M. sympodialis* has been frequently found more susceptible to the tested antifungals (Miranda *et al.*, 2007). The susceptibility of the yeast isolates to antifungal agents via a disk diffusion methodology was assessed following disk diffusion guidelines and interpretive zone sizes based on the CLSI standard. Certain aspects of this guideline were modified to overcome problems associated with the

colony characteristics, slower growth and incubation requirements of *Malassezia* species compared to *Candida* species in that: 1) Finding a suitable growth medium for *Malassezia*, especially the lipophilic species. This growth medium is often supplemented with a dispersing agent (mild detergent) to overcome the problem of cellular clumping which occurs due to the butyrous nature of this yeast. A dilute suspension of Tween 80 was added to the inoculum to act as a surfactant. 2) Increasing the inoculum size to counteract the slower growth rate of *Malassezia* compared to that of *Candida* species. The inoculum density was increased in order to produce semi-confluent growth. 3) The use of sterile glass beads and vigorous and continuous vortexing was essential to produce an even inoculum suspension. 4) Increasing the incubation time to up to 72 hours, again to counteract the slower growth rate of *Malassezia* compared to that of *Candida* species.

The present study revealed that the skin swabs were ideal clinical specimen for both cytological examination and isolation of *Malassezia pachydermatis*. Cultural studies show that SDA medium can be preferred over MDA for the isolation of *Malassezia pachydermatis*. Biochemical tests performed in isolates further confirmed for *M. pachydermatis* from the cases of canine dermatitis. Culture evidence indicated that only 41% of the cases were positive whereas 61% of cases were positive for *Malassezia* infection by PCR assay. Age-wise occurrence of *Malassezia*, the highest was noticed in one to three year old age dogs; Breed-wise occurrence, the highest was markedly prominent in Non-descriptive dogs and sex-wise occurrence, the highest incidence observed in male dogs than females. The frequency of isolation was higher in neck region followed by other regions in the dogs. As per the antifungal susceptibility test

performed; antifungal agents like Amphotericin B, Ketoconazole, Itraconazole and Fluconazole drugs can be suggested for the treatment of *Malassezia* dermatitis. Moreover, the study reveals that the drugs like Nystatin should not be recommended for the treatment of *M. pachydermatis* dermatitis in canines.

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