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Isolation and molecular identification of some *Malassezia* spp. isolated from dogs and human infected with pityriasis versicolor in Diyala Province

A Thesis
Submitted to the Council of the College of Veterinary Medicine, University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Microbiology

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Dedication

To Whom Who sent to us as a merciful prophet ((Mohammed)) peace be upon him. . . . . .

To that which was and is still the haven of national grief, my dear country ((Iraq)).

To those who light the way for science. . . . . .

To whom who taught me the letter ((My dear teacher)). . . . . .

To the candle that burned to lighten the way ((My beloved mother)). . . . . .

To whom who planted the seed of patience in myself and walked in my long way ((My darling father)). . . . . .

To the only branch in the garden of my life ((My beloved brothers, sisters and my wife)) and his flowers ((Saad, Mohamed, Mustafa and Aslam)) . . . . . .

To my dear uncle Deia Awad . . . . . .

With my loyalty and commitment

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Summary

Malassezia genus represents a group that is present in two distinct forms, normally habitat the skin of human and animals. However, it is implicated in various diseases, including Pityriasis Virsicolor (PV).

Fifty skin scraping samples from patients with PV in Diyala Province during the period (from 14 of October 2017 to 13 of January 2018) were included. Fifty skin scraping and ear swabs from dogs infected with skin diseases were collected. Specimens were examined by 10% KOH ,cultured on Sabouraud Dextrose Agar ,modified Dixons agar and *Malassezia* spp. identified according to morphological and biochemical tests. Genomic DNA was isolated from fungal growth and universal primers ITS3 and ITS4 were utilized to confirm the isolates .

The frequency of *Malassezia* spp. isolated from human was 27/50 (54%) ,while from dogs 31/50 (62%).This study showed that *M. furfur* was isolated from (32%) ; *M .globosa* (14%) and *M. pachydermatis* (8%) of PV patients. Male was more frequently infected with *Malassezia*, (40%). *M. furfur* was the predominant species recovered from males with PV cases,(22%) while *M. globosa* , (10%) and *M. pachydermatis* (8%). The age group (15-22)years was more exposed to Malassezia infection, while the age group (39-42) years was the minimal. Patients with primary education were more exposed to *Malassezia* infection, (22%). Illiterate patients were less exposed to *Malassezia* infection, (4%). Inverse negative correlation between age and exposure to *Malassezia* infection was reported. Positive correlation between gender and exposure to *Malassezia* infection was reported (p value =0.05) . Females have (2.619) time for getting *Malassezia* infection than male. No significant correlation was found between residence and *Malassezia* infection. Patients with rural residency have (1.09) time for getting *Malassezia* infection than urban. No significant correlation was found between economic status and *Malassezia* infection. Positive correlation between education status and *Malassezia* infection(p value =0.05). No significant correlation between job ,water source, contact with dogs and birds and *Malassezia* infection.

M. pachydermatis was isolated from,(62%) of dogs in which (18%) was isolated from otitis externa cases and (44%) from dermatitis cases. The mean age of dogs was (2.3 ±1.11) year . The high frequency of *M. pachydermatis* infection , (22%) was reported among dogs at 1-2 years old, while the least one among dogs at

3-4 years (14%). The high frequency of *M. pachydermatis* infection was reported among male dogs , (28%), and the high frequency of *M. pachydermatis* infection was reported among domestic dogs, (30%), Neither significant difference nor correlation was reported between age group, gender, dog breed and *M. pachydermatis* infection . No significant correlation between clinical signs appeared such as headshaking , head tilting , rubbing against wall , blackish waxy discharge, malodorous, erythematous lesion, unilateral ear drooping and malassezia infection in dogs. Positive correlation was reported between erythematous ceruminous and isolation of *M. pachydermatis*.

Pityriasis virsicolor due to *M. furfur; M. pachydermatis* and *M. globosa* appear to be quite common .PCR is very critical for confirmatory diagnosis of *Malassezia* infection. *Malassezia* infection inversely correlated with patients age; positively correlated with patients gender especially females and education. Residency in rural area and middle economic increase the possibility of infection. Infection is not affected by source of water ; job, contact with dogs and birds. regarding dogs, *M. pachydermatis* infection is not correlated with age group, gender or dog breed. Isolation of *M. pachydermatis* is correlated with erythematous ceruminous .

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List of abbreviations

Abbreviation	Meaning
AD	Atopic dermatitis
AFLP	Amplified fragment length polymorphism
AhR	Arylhydro carbon receptor
bp	base pair
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribo nucleic acid
GYP-S	Glucose-Yeast-Pepton-Supplement
ICZ	Indolo carbazole
ITS	Internal transcribed spacer
KOH	Potassium hydroxide
Lab	Laboratory
M	*Malassezia*
mDA	modified Dixon's Agar
PCR	Polymerase Chain Reaction
PFGE	Pulsed-field gel electrophoresis
pH	power of Hydrogen
PSI	pound per square inch
PV	Pityriasis versicolor
RAPD	Random amplified polymorphic
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
rpm	rounds per minute
RT	Room temperature
SC	Stratum corneum
SDA	Sabouraud Dextrose Agar
Sig	significant
spp.	species
SPSS	Statistical Package for Social Sciences
TNF	Tumor necrosis factor
UV	Ultra violet
µ	Micro

Chapter One

Introduction

1.1. Introduction:

Malassezia spp. a lipophilic unipolar budding yeasts characterized by a thick cell wall , are commensal skin organisms of warm- blooded vertebrates **(Cafarchia *et al.*, 2011).** They are opportunistic pathogens that may cause systemic and skin disorders in human and animals. They are involved in the aetiology of some important skin disorders including Pityriasis versicolor (PV), folliculitis , seborrheic dermatitis and dandruff , psoriasis , confluent reticulate papillomatosis and seborrheic blepharitis **(Al-Ezzy *et al.*, 2017).**

Members of the genus *Malassezia* cause different manifestations in dogs including dermatitis and otomycosis and as a possible aggravating factor in the physiopathology of corneal ulcers **(Ashbee, 2007;Sihelská *et al.*, 2017).**

Pityriasis versicolor is a chronic superficial fungal infection that appears as flat, slightly scaly discolored patches on the upper trunk, neck, and upper. arms **.** The word "versicolor" implies that this rash can have several different colors , and indeed the eruption may be lighter or darker than a person's normal skin or even have a reddish appearance **(World Health Organization, 2018) .**

The organism's yeast phase shows two morphologically distinct forms, one ovoid, the other spherical, in which the fungus is named *Pityrosporum ovale* and *Pityrosporum orbiculare* respectively . PV is also known as tinea versicolor, dermatomycosis furfuracea and tinea flava **(Rai and Wankhade, 2009) .**

Pityriasis versicolor is one of the most common dermatomycosis , and is especially prevalent in regions with a warm humid climate , where up to 40 % of the population may be affected , PV is the

prototypical skin disease etiologically connected to *Malassezia* species **(Gaitanis *et al.*, 2012) .**

The use of molecular methods has revolutionized the study of disease due to *Malassezia* species because the organisms are fastidious and difficult to identify. The introduction of a new taxonomy in 1996 led to a series of revealing studies of the etiology of diseases linked to *Malassezia* species **(Hay and Jones, 2010) .**Colony morphology and biochemical characters can be used for the identification of *Malassezia* species **(Puig *et al.*, 2017).** DNA based technique was utilized for the identification of *Malassezia* instead of phenotypic methods to reduce the time required for the diagnosis and lack of sufficient discrimination between newly identified species **(Böhmová *et al.*, 2018) .**

1.2. Aims of the study

The study aims at the :

[1] Isolation of *Malassezia* spp. from pityriasis versicolor patients in Diyala province .

[2] Isolation of *Malassezia* spp. from Dogs with otitis externa and dermatitis in Diyala province .

[3] Identification of *Malassezia* spp. by phenotypic characterization , biochemical tests and PCR based molecular technique .

[4] Evaluation of relationship between *Malassezia spp.* infection and possible risk factors in human and dogs.