

Phenotyping And Genotyping Identification Of Important Human, Animal And Soil Of Dermatophytes

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ABSTRACT

In the current study, 62 dermatophyte isolates obtained from human and animal dermatophytosis as well as from soil were subjected for phenotypic and genotypic identification. The conventional (phenotypic) method for identification of dermatophytes in the present work was succeed to identify all isolates through macro-morphology, micro-morphology and differential media into species.

Identification of 48 isolates obtained from human cases revealed 15 *M.canis*, 12 *T.violaceum*, 12 *T.rubrum*, 5 *E.fluccosum* and 4 *T.mentagrophytes*. The eight isolates obtained from cattle were identified as *T.verrucosum*, while the four isolates obtained from dogs and cats were identified as *M.canis*. On the other hand, two dermatophyte isolates obtained from soil were identified as *M.gypseum*.

On other hand, three methods were used for molecular (genotypic) identification of dermatophytes which include: a) PCR for amplification of ITS1 and ITS4 followed by restriction fragment length polymorphism (RFLP) using *MvaI* for 30 isolates of dermatophyte formerly identified by phenotypic method. b) Application of PCR using single repetitive oligonucleatede (GACA)₄ for 30 dermatophyte isolates formerly identified by phenotypic and RFLP methods c) DNA sequencing which done for 5 representative isolates of *M.canis*, *T.verrucosum*, *T.violaceum* and *T.rubrum*.

While RFLP using *MvaI* method and repetitive(GACA)₄ method identified the thirty dermatophyte isolates into species identical to those identified by phenotypic methods, sequencing identified one isolate formerly identified by phenotypic, *MvaI* and (GACA)₄ as *T.rubrum* with similarity 99% as *T.raubitschekii*.

Although molecular methods are rapid and represents technological advance in the laboratory diagnosis, it is expensive. So we recommended its use in absence of skilled mycologist, in identification of atypical or variants of dermatophyte species.

INTRODUCTION

Dermatophytes are the main cause of superficial mycoses. These fungi have the capacity to invade keratinized tissue of humans or animals to produce dermatophytosis (ringworm) that are generally

restricted to the corneocytes of the skin, hair, and nail. Routine procedures for dermatophyte species identification rely on macroscopic examination of the colony (pigmentation of the surface and reverse sides, topography, texture, and rate of growth) and microscopic morphology (size and shape of macro conidia

and microconidia, spirals, nodular organs, and pectinate branches) (1).

The etiologic agents of dermatophytosis are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum* and *Trichophyton*. The descriptions of the genera essentially follow the classification scheme of Emmons (2) on the bases of conidial morphology and formation of conidia and are updated following the discovery of new species (3).

Dermatophytes and their congeners have long been divided into anthropophilic, zoophilic and geophilic species on the basis of their primary habitat associations (4). Anthropophilic dermatophytes are primarily associated with humans and rarely infect other animals (5). Zoophilic dermatophytes usually infect animals or are associated with animals but occasionally infect humans. Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves and horns after these materials have been dissociated from living animals and are in the process of decomposition (6).

Dermatophyte identification is usually depending on conventional methods based on detection of phenotypic characteristics such as microscopy and in vitro culture (7). Morphological and physiological characteristics can frequently vary, in fact, the phenotypic features can be easily influenced by outside factors such as temperature variation and medium (8).

In the last few years genotypic approaches have proven to be useful for solving taxonomic problems regarding dermatophytes. Genotypic differences are considered more stable and more precise than phenotypic characteristics (9). Molecular techniques are more beneficial for dermatophyte identification as they are rapid and more sensitive. Moreover, these methods rely on genetic makeup, which is more constant than phenotypic characterization, and they can identify atypical dermatophytes that could not be identified by culture based techniques. These genotypic approaches can

identify dermatophytes into species as well as the strain levels (10,11). Molecular methods, such as, PCR (random amplification of polymorphic DNA (RAPD) (7), arbitrarily primed PCR (AP-PCR) (12) and PCR fingerprinting (13) have brought important progress in distinguishing between species and strains (14).

The present work was done to study methods of identification of dermatophytes from human, animal and soil by conventional and genotypic methods.

MATERIAL AND METHOD

Samples

Human samples: Fifty five skin scrapings and hair were collected from patients suffering from dermatophytosis and attending to Out Patient Clinic of Dermatology at Misr University for Science and Technology (MUST) and private laboratory for mycology investigation (Cairo) between May 2010 to September 2011. The cases include 25 tinea capitis, 15 Tinea corporis, 10 Tinea cruris and 5 tinea pedis.

Ten skin scrapings and hair samples were collected from cattle showed clinical signs of ringworm at private farms in Zagazig Providence. Five skin scraping and hair samples were collected from 2 dogs and 3 cats showed ringworm at private veterinary clinic and owner's. Five soil samples contaminated by animal wastes were collected at El Sharkia Governorate from soil.

Collection of samples: after treated the lesions with 70% ethyl alcohol, skin scrapings were collected from each case in sterile Petri-dish (6 mm) by using new sterile blade scalpel for each case. Hairs were collected by forceps.

Direct microscopic examination was done after use of KOH, DMSO, orcalcofluore

white (Remel) which used for direct microscopic examination for fungal elements by light or fluorescent microscope.

Cultivation of the specimens

Skin scraping and hair from human and animal samples were inoculated in the following media: Dermasel agar, DTM, Derm-Duet, and In-Try DM (All inoculated media were incubated at 30°C for two weeks.

Hair-bait technique (19)

Soil sample placed on Petri dish then covered by sterilized short hair (3-5 cm). Drops of mixture of cycloheximide and chloramphenicol were added then incubated at room temperature for 10-15 days. The growth transferred to Dermasel agar.

Identification of isolated dermatophyte

Phenotypic identification (20-24)

- Macro-morphological examination: Growth rate, consistency, surface color, reverse color as well as change of DTM and in-Try DM color were examined every three days of inoculation until the end of incubation.
- Micro-morphological examination: in case of In Try DM, the cartilage was examined through clear viewing window under microscope for hyphae and conidia, in case of other media particles from the growth were added on slide with drops of LPCB then overlaid by cover slip and examined under microscope for modification of hyphae, macroconidia and microconidia.
- Cultivation on differential media: subculture of the isolated dermatophyte was done on the following media, BCP, LA, MHB and RG (21-24).

All inoculated media were examined for rate of growth, surface and reverse color of the growth and changes of the medium. Sporogenous effect of the media was examined by microscopic examination of the colonies after LPCB.

Genotypic identification of the isolates

Thirty isolate from 7 species of dermatophytes identified by phenotypic methods were subjected for PCR (Table 1).

Table 1. Samples subjected to PCR

Sample source	Human	Animal	Soil	Total
<i>T. verrucosum</i>	-	6	-	6
<i>M. canis</i>	4	3	-	7
<i>E. floccosum</i>	2	-	-	2
<i>T. rubrum</i>	4	-	-	4
<i>T. mentagrophytes</i>	2	-	-	2
<i>T. violaceum</i>	7	-	-	7
<i>M. gypseum</i>	-	-	2	2
Total	19	9	2	30

Kits for DNA extraction: DNA extraction from isolated dermatophyte was done using DNeasy plant Mini kit Qiagen Genomic Protocol extraction kit as described by manufacturer manual of Qiagen, Germany.

Primers: There are 2 types of primers were used

General primer (11)

Forward (ITS1) 5'-TCCGTAGGTGAACCTGCGG-3'.

Reverse (ITS4) 5' TCCTCCGCTTATTGATATGC-3'.

Repetitive primer (GACA) 4 (11)

5'-GACAGACAGACAGACA-3'

Gene Sequencing was done by Lab Technology Korea for 5 representative isolates {*T. violaceum* (1), *M. canis* (1), *T. verrucosum* (1) and *T. rubrum* (2)}.

RESULTS

Direct microscopic examination

In human cases positive microscopic examination (KOH) was detected in 92.72%. While cattle ringworm cases revealed positive KOH in 100%, 80 % ringworm cases of dogs and cats were positive KOH.

Isolation of dermatophytes from examined samples

- Dermatophytes were isolated from 48 out of 55 human cases on Dermasel agar and DTM, while In-Try DM and Derm-Deut failed to isolate many of them.
- Dermatophytes were isolated from 8 out of 10 cases of cattle ringworm.
- Dermatophytes were isolated from 4 out of 5 dogs and cats, four isolates (one from dog and 3 from cats).
- Dermatophytes were isolated from 2 out of 5 soil samples.

Table 4 showed the results of cultivation on different media used for isolation of dermatophytes from human, animals and soil. Dermasel agar and DTM were suitable for isolation of human dermatophytes while In-Try DM and Derm-Deut failed in isolation of some of them. Dermasel and DTM were the most suitable for isolation of dermatophytes from cattle. All media succeeded in isolation of dermatophyte from dogs and cats as well as from soil. Figs.(1 and 2) showing macro and micromorphology of *M. canis* on In Try DM.

Table 2. Comparison between dermasel agar, DTM, In-Try DM and Derm-Deut in isolation of dermatophytes

Media/ source	Dermasel agar	DTM	In Try DM	Derm-Deut
Human	48	48	38	22
Cattle	8	8	-	-
Dogs and cats and cats and cats	4	4	4	4
Soil	2	2	2	2
Total isolates	62	62	44	28

C- Phenotypic Identification of dermatophyte

Macro and micro-morphological identification of dermatophytes isolates

With help of keys manual for identification of dermatophytes. Sixty and two isolates obtained from human, animals and soil were identified according to macro-morphology and micro-morphology as the following :

- Human: As showed in table (3) 48 dermatophyte isolates were identified as *M. canis* (15), *T. violaceum* (12), *T. rubrum* (12), *T. mentagrophytes* (4) and *E. Fluccosum* (5). While *M. canis* and *T. violaceum* were the causative agent of tinea capitis, *T. rubrum*, *M. canis* and *T. violaeum* were the causative of tinea corporis. On the other hand *T. cruris* caused by *E. fluccosum* and *T. rubrum*, and tinea pedis caused by *T. mentagrophytes*.

Table 3. Identified dermatophytes among 48 human samples

	<i>M.canis</i>	<i>T.violaceum</i>	<i>T.rubrum</i>	<i>T.mentagrophytes</i>	<i>E.fluccosum</i>
Tinea capitis	12	10	-	-	-
Tinea corporis	3	2	9	-	-
Tinea cruris	-	-	3	-	5
Tinea pedis	-	-	-	4	-
Total	15	12	12	4	5

b) Cattle: The eight isolates of dermatophytes obtained from cattle dermatophytosis were identified according to macromorphology and micromorphology into *T.verrucosum*.

c) Dogs and cats: Four isolates obtained from dogs and cats were identified as *M.canis*

d) Soil: Two isolates obtained from soil samples identified as *M.gypseum*.

Figs. (3, 4, 5, 6 and 7) showing the micromorphology of *M. canis*, *M.gypseum*, *T.mentagrophytes*, *T. rubrum* and *T. violaceum*.

Cultivation on differential media

Cultivation of dermatophyte isolates on RG,LA,PCP and MHB media confirmed the identification of dermatophyte after macro and micro- morphological identification. Figs. (8, 9, 10 and 11) showing the growth of dermatophytes on BCB, RA and MHB. Increase pigmentation, change of medium colour, hydrolysis of casein as well as increase conidiation were the tool used in this type of identification.

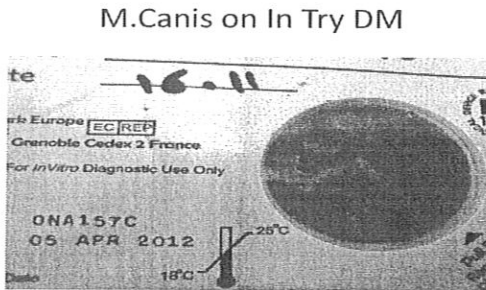


Fig. 1. *M. canis* on In Try DM hairy white colony and the medium turned red

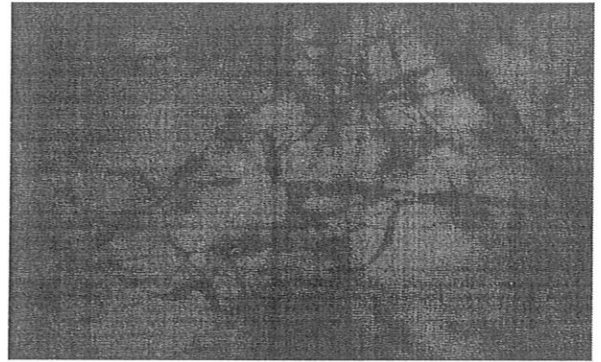


Fig. 2. Microscopic exam. of *M. canis* on In Try DM showing macroconidia

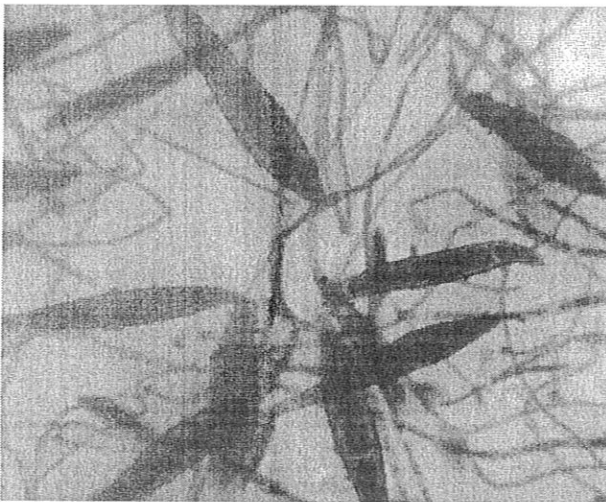


Fig. 3. *M. canis* showing macroconidia (LPCB)



Fig. 4. Microscopic examination of *M. gypseum* showing spindle shaped macroconidia (LPCB)

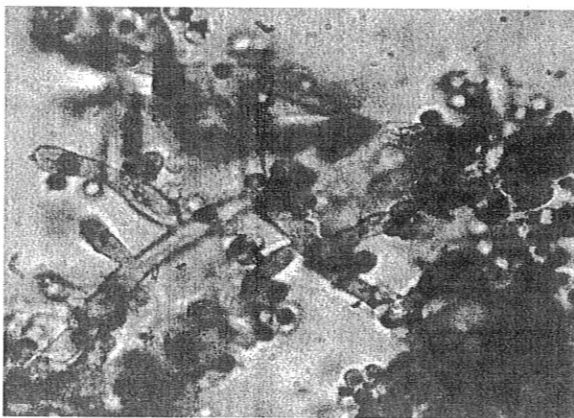


Fig. 5. Microscopic examination of *T. mentagrophytes* showing macroconidia and microconidia (LPCB)

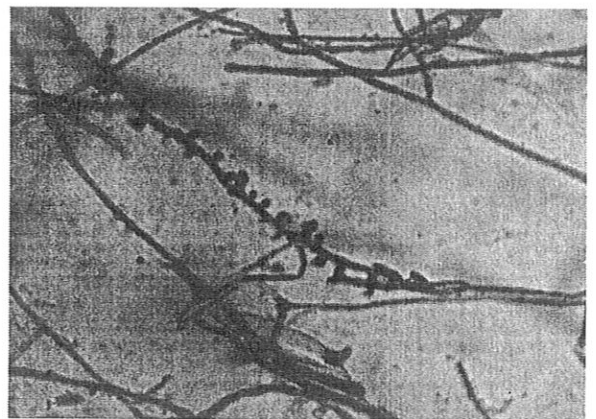


Fig. 6. Microscopic exam. of *T. rubrum* showing microconidia sessile on sides of hyphae (LPCB)



Fig. 7. Microscopic exam. of *T. verrucosum* showing chains of chlamydozoospores (LPCB)

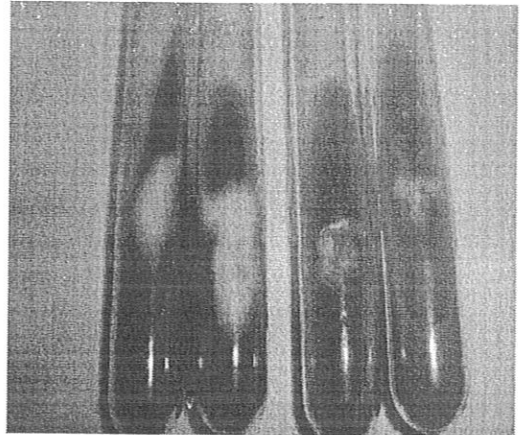


Fig. 8. BCP medium: left *T. mentagrophytes* turned the medium purple while right *M. canis* showing profuse growth without changing in color

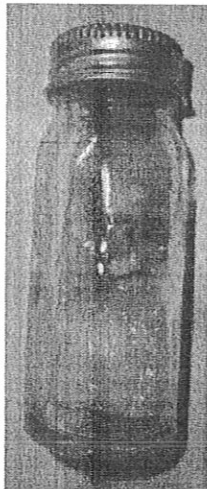


Fig. 9. *M. canis* on RG showing yellow pigmentation

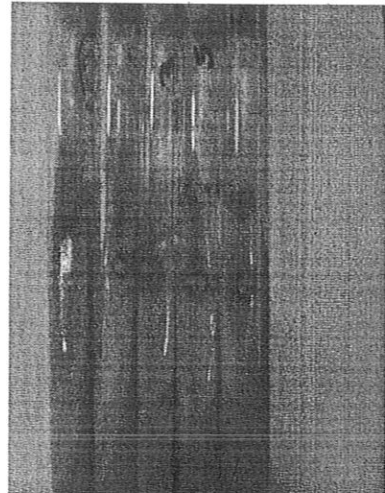


Fig 10. *T. verrucosum* on MHB with halo zone around colonies and green color (7 days).



Fig. 11. *T. verrucosum* on MHB (14 d) showing good growth with coloration of the medium

D.PCR results

In the present study three methods were used for molecular identification of dermatophytes which include:

- a) PCR for amplification of ITS1 and ITS4 by common primer followed by restriction fragment length polymorphism (RFLP) using MvaI.
- b) Application of PCR using single repetitive oligonucleotide (GACA)₄

- c) DNA sequencing which done only for 5 representative isolates.

Identification of dermatophyte isolates by common primer and RAPD

PCR with the ITS1/ITS4 primer set for 30 dermatophyte isolate resulting in amplified products of approximately a fragment of 690 bp specific for *T. violaceum*, *T. rubrum*, *T. verrucosum*, *M. gypseum* and *T. iolaceum* (Fig. 12,13 and 14 and 740 bp for *M. canis* (Fig .15).

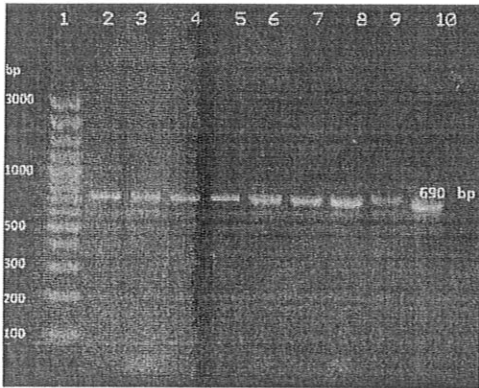


Fig. 12. Agarose gel electrophoresis *T. verrucosum* and *Microsporium gypsum* DNA products
Lane 1: Molecular weight marker,
Lane 2-7: *T.verrucosum* (cattle).
Lane 8-9: *Microsporium gypsum* (Soil)
Lane10: *E. floccosum*

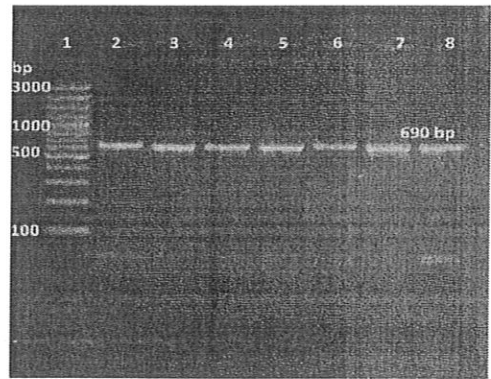


Fig. 13. Agarose gel electrophoresis *T. rubrum* and *T. mentagrophyte* DNA products
Lane 1: Molecular weight marker,
Lane 2-5:*T. rubrum* (human)
Lane 6-7: *T.mentagrophyte* (human)
Lane 8: *E. floccosum* (man)

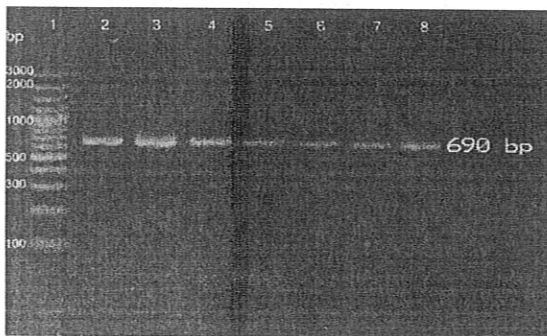


Fig. 14. Agarose gel electrophoresis for PCR product of the ITS1 and ITSII of the *T. violaceum*
Lane 1: Molecular weight marker,
Lane 2-8: *T. violaceum* strain (human)

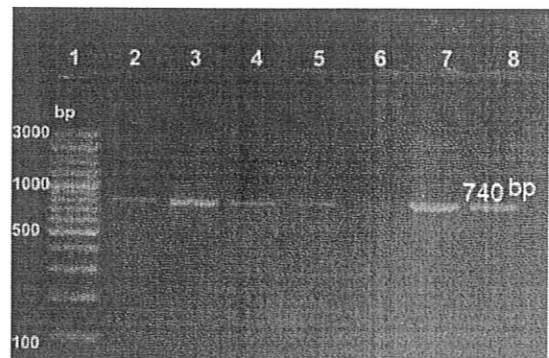


Fig. 15. Agarose gel electrophoresis *M. canis* DNA products
Lane 1: Molecular weight marker,
Lane 2-8: *M. canis* (Human)

MvaI digestion of these amplified products from each of the dermatophyte isolates revealed unique restriction patterns, with no interspecies variation. *M. canis* isolates showed three band patterns, ranging from 100 bp to 500 bp in size, with a marked

size difference between the largest and middle bands(Fig.16).

On the other hand, both *T. violaceum* and *T. rubrum* isolates resulted in four bands, with sizes ranging between 50 bp and 400 bp (Fig. 17).

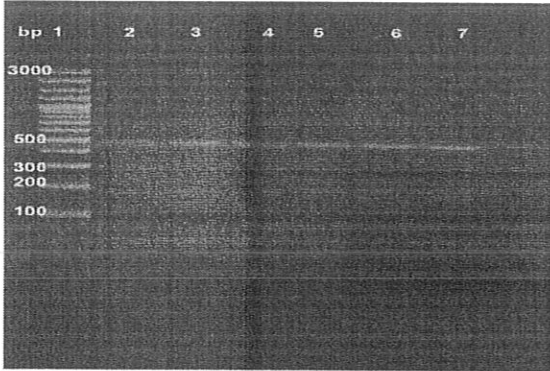


Fig. 16. Agarose gel electrophoresis of *MvaI* restriction products of *M. canis*
Lane 1: Molecular weight marker,
Lane 2-7: *M. canis* (Human)

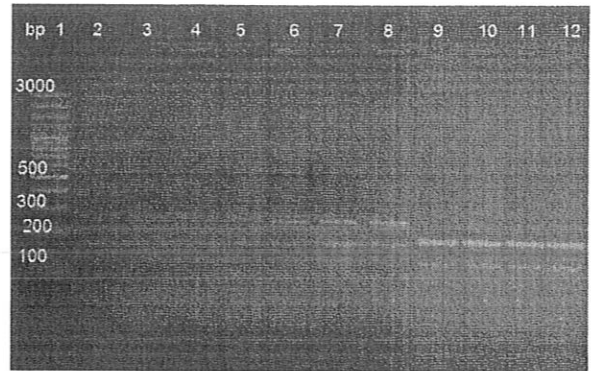


Fig. 17. Agarose gel electrophoresis of *MvaI* restriction products of *T. violaceum* and *T. rubrum*
Lane 1: Molecular weight marker,
Lane 2-8: *T. violaceum*
Lane 9-12: *T. rubrum*

MvaI restriction products of *T. verrucosum*, *M. gypsum*, *T. mentagrophytes* and *E. floccosum* showed fourband patterns its

sizes ranging between 100 bp and 500 bp (Fig.18).

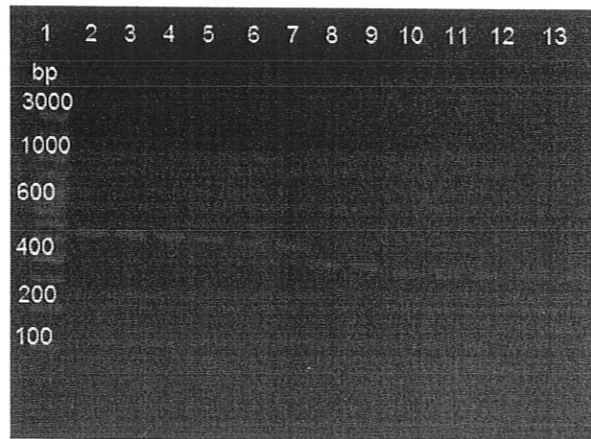


Fig. 18. Agarose gel electrophoresis of *MvaI* restriction products of *T. verrucosum*, *M. gypsum*, *T. mentagrophyte*, *floccosum*

(a) Identification of dermatophyte by (GACA) 4 based PCR

Thirty dermatophyte isolates were amplified with (GACA)₄. The results showed that the numbers of PCR bands ranged from 9 to 13 (size range, 200 bp to 1300bp).

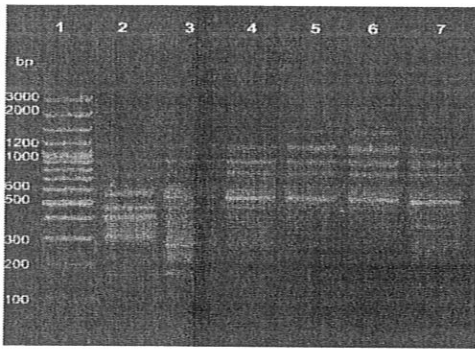


Fig. 19. Agarose gel electrophoresis DNA products
Lane 1: Molecular weight marker,
Lane 2-6: *T. verrucosum* (cattle)
Lane 7: *T. verrucosum* (Human)

T. verrucosum (6 isolates) revealed banding patterns ranging from 177 bp to 1,240 bp in size, with one strong band of 628 bp. (Fig.19).

(GACA)₄-based PCR of *M. canis* strains revealed the most complex profiles, with up to 11 bands, ranging from 170 bp to 1,200 bp in size. There was no interspecies variation among *M. canis* isolates, all of which had the same band pattern (Fig. 20).

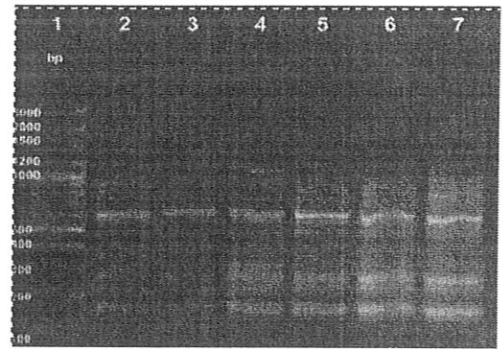


Fig. 20. Agarose gel electrophoresis DNA products using GACA primer
Lane1: DNA ladder,
Lane 2-7: *M. canis* (Human)

Profiles for *M. gypsum*, and *T. rubrum* were more complex, in *M. gypsum* comprising eleven or twelve bands, between 160 bp and approximately 1,200 bp in size. In *T. rubrum* we found five to twelve bands ranged from 200 to 2000 bp. (Fig.21).

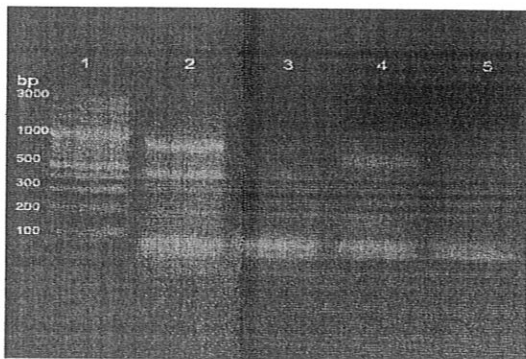


Fig. 21. Agarose gel electrophoresis of DNA products of Dermatophytes isolates
Lane1: Molecular weight marker,
Lane 2-3: *E. floccosum*,
Lane 4-5: *T. mentagrophytes*

DNA sequencing

While, *M. canis*, *T. verrucosum*, *T. violaceum* and one isolate of *T. rubrum* were found to be identical to similar in Gen Bank, the other isolate of *T. rubrum* obtained from a case of human tinea corporis was found to be identical (99 %) for the sequence of the *T. raubitscheki* (Figs.14,15 and 16) and table (4).

Table 4. Sequencing of five dermatophyte isolates relate to 4 species

S. N.	Description	Max score	Total score	Max ident
1 F	<i>Trichophyton violaceum</i> isolate UOA/HCPF13250 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	304	356	97%
1 R	<i>Trichophyton violaceum</i> isolate UOA/HCPF13250 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	304	401	94%
2 F	<i>Microsporum canis</i> strain ATCC MYA-4605 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	880	880	99%
2 R	<i>Microsporum canis</i> strain ATCC MYA-4605 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1227	1227	98%
3 F	<i>Trichophyton verrucosum</i> 5.8 rRNA gene and ITS1 and ITS2 DNA (strain CBS 134.66)	1188	1188	99%
3 R	<i>Trichophyton verrucosum</i> 5.8 rRNA gene and ITS1 and ITS2 DNA (strain CBS 134.66)	1184	1187	99%
4 F	<i>Trichophyton rubrum</i> 5.8 rRNA gene and ITS1 and ITS2 DNA (strain CBS 392.58)	1085	1488	96%
4 R	<i>Trichophyton rubrum</i> 5.8 rRNA gene and ITS1 and ITS2 DNA (strain CBS 392.58)	1184	1184	99%
5 F	<i>Trichophyton raubitschekii</i> strain BMU04349 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1134	1134	99%
5 R	<i>Trichophyton raubitschekii</i> strain BMU04349 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1134	1134	99%

From the above mentioned results we become confident to the similarity of genotypic result and phenotypic result except in *T. rubrum* which has similarity in some

morphological and physiological characters with *T. raubitschekii* which considered homologue genetically with *T. rubrum* (Figs. 22&23).

Sequence ID: gb|EU921293.1|Length: 774Number of Matches: 1

Related Information

Range 1: 153 to 774GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

	Score	Expect	Identities	Gaps	Strand
	1134 bits (614)	0.0	622/625 (99%)	3/625 (0%)	Plus/Plus
Query 4	AACGGCGCAGGGCCGTGAGGCTGGCCOCCCCACGATAGGGACCGACGTTCCATCAGGGGTGA				63
Sbjct 152	AAC-GCCGAGGCCG-GAGGCTGGCCOCCCCACGATAGGGACCGACGTTCCATCAGGGGTGA				210
Query 64	GCAGACGTGCGCCGGCCGTACGCCOCCCATTCITGTCTACCTCACCCGGTTGCCCTCGGCCG				123
Sbjct 211	GCAGACGTGCGCCGGCCGTACGCCOCCCATTCITGTCTACCTCACCCGGTTGCCCTCGGCCG				270
Query 124	GCCGCGCTCCCCCTGCCAGGGAGAGCCGTCGGCGGGCCOCCCTTCYGGGAGCCTCGAGCCG				183
Sbjct 271	GCCGCGCTCCCCCTGCCAGGGAGAGCCGTCGGCGGGCCOCCCTTCYGGGAGCCTCGAGCCG				330
Query 184	GACCGCGCCCCGCCGGAGGACAGACACCAAGAAAATTCTCTGAAGAGCTGTCAGTCTGA				243
Sbjct 331	GACCGCGCCCCGCCGGAGGACAGACACCAAGAAAATTCTCTGAAGAGCTGTCAGTCTGA				390
Query 244	GCGTTTAGCAAGCACAAATCAGTTAAAATTTTCAACACGGATCTCTTGGTTCCGGCATCG				303
Sbjct 391	GCGTTTAGCAAGCACAAATCAGTTAAAATTTTCAACACGGATCTCTTGGTTCCGGCATCG				450
Query 304	ATGAGAAACCGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATCCCGTGAATCATCGA				363
Sbjct 451	ATGAGAAACCGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATCCCGTGAATCATCGA				510
Query 364	ATCTTGAACGCACATTGCCOCCCTCTGGCATTCCGGGGGGCATGCCTGTTCCGAGCGTCAT				423
Sbjct 511	ATCTTGAACGCACATTGCCOCCCTCTGGCATTCCGGGGGGCATGCCTGTTCCGAGCGTCAT				570
Query 424	TTCAACCCCTCAGCCCCGGCTTGTGTGATGGAGACCGTCCGGCCOCCCTCCCTTCGGGGGC				483
Sbjct 571	TTCAACCCCTCAGCCCCGGCTTGTGTGATGGAGACCGTCCGGCCOCCCTCCCTTCGGGGGC				630
Query 484	GGGACGCGCCCCGAAAGCAGTGGCCAGGCCGCAATCCGGCTTCCTAGGCCGAATGGGCAG				543
Sbjct 631	GGGACGCGCCCCGAAAGCAGTGGCCAGGCCGCAATCCGGCTTCCTAGGCCGAATGGGCAG				690
Query 544	CCAATTCAGCGCCCTCAGGACCGGGCCGCCCTGGCCCCAATCTTtatatatatatatatCT				603
Sbjct 691	CCAATTCAGCGCCCTCAGGACCGGGCCGCCCTGGCCCCAATCTTtatatatatatatatCT				750
Query 604	TTTCAGGTTGACCTCGGATCAGGTA 628				
Sbjct 751	TTTCAGGT-GACCTCGGATCAGGTA 774				

Fig. 22: Forward sequencing for sample number 5.

DISCUSSION

Conventional methods for dermatophytes identification are based on detection of fungal elements by direct microscopy of clinical specimens combined with culture-based identification including macro-morphological, micro-morphological and physiological characters of the colonies. In the last few years, genotypic approaches have been proven to be useful for solving identification problems regarding dermatophytes. Genotyping differences are considered more stable and more precise than phenotypic identification characteristics (25-27).

The aim of the present study was done to correlate between conventional and molecular methods for dermatophyte isolates obtained from human, animals and soil.

Fifty five human samples and 15 animal samples (cattle, dogs and cats) collected from cases of dermatophytosis as well as 5 soil samples were subjected for mycological examination.

While human specimens gave positive direct microscopic examination (KOH) in 92.72 % and positive culture in 87.27%, animal specimens gave positive KOH in 93.33% and 80% positive culture. Similar results were recorded by previous authors (17, 28,29) who contributed the higher positivity of KOH than culture due to; the cases were under treatment, the culture contaminated by rapid growing fungi giving no chance for slowly growing dermatophytes to be appear or even the use of unsuitable media for certain dermatophyte with higher nutritional requirements. In general KOH and culture complete each other and while direct microscopic examination showed higher sensitivity, culture showed higher specificity as mentioned (30).

Comparison between four media namely; Dermasel agar, DTM, In-Try DM and Derm-Deut, it was found that Dermasel and DTM succeeded in isolation of 62 dermatophyte isolates from total of 75 specimens while from In-Try DM and Derm-

Deut 44 and 28 dermatophyte isolates obtained respectively. The reason why the two ready prepared media failed to catch up all dermatophyte isolates may be due to, the media subjected to dryness before the growth of slow growing dermatophytes as *T.violaceum* and *T.verrucosum* and plates of Derm-Deut subjected rapidly for contamination by non dermatophyte moulds. These results coincide with (28) who obtained high rate of dermatophytes on Dermasel agar and with (31) who found that the usefulness such in-office culture systems is still a matter of debate.

Phenotypic identification of dermatophyte species rely on macro-morphology of colonies (rate of growth, texture and colour of surface and reverse side), and micro-morphology (presence and characters of macroconidia and microconidia as well as modification of hyphae e.g. spirals, nodular organs, favic chandeliers and pectinate bodies) and when identification of them not reached, biochemical tests such as urease, nutritional requirements and in-vitro hair penetration test will help in its identification (32,33).

On the other hand, besides rice grain medium which used for differentiation of *M.canis* from *M.audouini* (18), bromocrusol purple medium (34) for differentiation of *T.mentagrophytes* from *T.rubrum*, other differential media were propagated (16,17,35,36) for differentiation between dermatophytes confused in their morphological and physiological characters.

In the present work 62 dermatophyte isolates obtained from human and animals dermatophytosis as well as from soil were subjected for macro-morphology, micro-morphology examination and culture on four differential media (RG, LA, BCP and MHB).

The identification of 48 isolates obtained from human cases revealed 15 isolates of *M.canis*, 12 of *T.violaceum*, 12 of *T.rubrum*, 5 of *E.fluccosum* and 4 of *T.mentagrophytes*. These results are in accordance with those published (17,36) who found that *M.canis* was

the predominant dermatophyte isolated in last few years in Egypt followed by *T.violaceum* and *T.rubrum*, while differ from (38) who isolated *T.violaceum* 67% from cases of tinea capitis in Sharkia Governorate which may be due to the variation of results according to the place where the study were done.

Concerning with identification of dermatophytes isolated from animals, it was found that while *T.verrucosum* was the only dermatophyte isolated from cases of cattle, *M.canis* was the only dermatophyte isolated from dogs and cats. Similar results were recorded (39,40) who pointed out that *T.verrucosum* is the major dermatophyte encountered in cattle and with (41,42) who considered *M.canis* as the dominant species in dogs and cats.

The two dermatophyte isolates obtained from 5 soil samples were identified as *M.gypseum*, the result which is in agreement with (43).

The conventional (phenotypic) method for identification of dermatophytes in the current study were succeed to identify all isolates through macro-morphology and micro-morphology into species which confirmed by the four differential media used

Nucleic acid based technique relies on the genotypic differences in pathogenic organisms. They are intrinsically more specific and more precise than those based on phenotypic features (44).

Recently a number of methods have been reported for molecular identification of dermatophytes which include:

- 1-Polymerase chain reaction targeting 18s Rdn (45).
- 2-Random amplified polymorphic DNA [RABD] (46).
- 3-PCR using single simple repetitive oligonucleotide (GACA)₄ (7)
- 4-Restriction fragment length polymorphism [RFLP] (47).

5- Chitin synthesis I (CHSI) gene sequencing (15).

6- Arbitrary primed PCR [AP-PCR] (7).

On the otherhand, PCR immunosorbant assay[PCR-ELISA], line block PCR[PCR-RLP] and multiplex real time PCR are propagated for detection of dermatophytes in clinical materials (48, 49,50).

In the present study three methods were used for molecular identification of dermatophytes which include;

- a) PCR for amplification of ITS1 and ITS4 followed by restriction fragment length polymorphism (RFLP) using *MvaI* for 30 isolates of dermatophyte formely identified by phenotypic method.
- b) Application of PCR using single repetitive oligonucleatede (GACA)₄ for 35 dermatophyte isolates identified by phenotypic and RFLP methods.
- c) DNA sequencing which done only for 5 representative isolates.

Amplified products using universal primers ITS1and ITS4 from *T.violaceum*, *T.rubrum*, *T.mentagrophytes* and *E.fluccosum* were found at 690 bp while *M.canis* was at 740 bp, the result which identical (11).

MvaI digestion of amplified products in the first step revealed unique restriction pattern. Analysis of number and size of patterns identified the 35 isolates examined of dermatophytes into species typical to those with phenotypic method. The results of current work by RFLP are coinciding with (51) who found that PCR-RFLP of ITS region was easily identifiable fragment patterns for all dermatophyte isolates into species.

Concerning with (GACA)₄-based PCR in the current study, it was found that all isolates were identified by it into identical manner as phenotypic as well as PCR-RFLP methods. The present study is in agreement with those done by (7,52) when they found that repetitive primer (GACA)₄ was able to amplify all dermatophytes into species. Comparing the two methods the RFLP is

complex, needing much effort and time while (GACA) 4 method is simple and rapid.

While the two methods of genotypic in the present study are in agreement in 100% with the results of phenotypic methods. Identification of dermatophyte by the APC-PCR was in agreement with the phenotypic methods in 86.8% of the isolates. It may be due to shortage in phenotypic identification as lack experience in identification of dermatophytes and use of macro-morphology and micro-morphology without help of other methods as differential media (28).

On the other hand five dermatophyte isolates [*M.canis* (1), *T.violaceum* (1), *T.verrucosum* (1) and *T.rubrum* (2)] formerly identified by RFLP and repetitive primer (GACA)₄ were sent to sequencing then the data were analyzed by DNA software comparing to those in Gen Bank. While *M.canis*, *T.verrucosum*, *T. violaceum* and one isolate of *T.rubrum* were found to be identical to similar in Gen Bank, the other isolate of *T.rubrum* obtained from a case of human tinea corporis was found to be identical (99%) for the sequence of the *T.raubitscheki*, a dermatophytes considered as atypical dermatophyte confused of *T.rubrum* and rarely isolated from people who live or traveled in Mediterranean (53,54). While it differ from *T.rubrum* in some morphological and physiological characters specially urease test which is positive in 3 days, *T.raubitschekii* is homologue genetically with *T.rubrum* (55). It is the reason why RAPD and (GACA)₄ not differentiate them in the present work.

Although sequencing provides a very accurate and useful method for the identification of dermatophytes, it is highly expensive to use in routine genotypic identification the reason why (56) recommended its use for identify a minority of atypical isolates of dermatophytes.

The results in the current study revealed the capacity of phenotypic (morphological and differential media) and genotypic (RFLP) and repetitive primer) in equally manner to identify the isolates of dermatophytes obtained from

human, animals and soil. Although molecular methods is rapid and represents technological advance in laboratory diagnosis, it is expensive and facing some problems in our country. So we recommended its use in identification of dermatophytes for atypical and variant of species.

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المخلص العربي

دراسات مقارنة بين طرق الاطر المظهرية والاطر الوراثة لتشخيص مرض الفطريات الجلدية للانسان والحيوان

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في الدراسة الحالية تم تجميع عدد ٦٢ عينة من الفطريات - تم الحصول عليها من الالتهابات الجلدية الفطرية من الإنسان والحيوان وكذلك من التربة وتم عمل الاختبارات التقليدية (المظهرية) والوراثية. حيث تم التعرف بالطريقة التقليدية على جميع العينات من خلال الشكل الظاهرى للفطريات وكذلك الشكل تحت الميكروسكوب وباستخدام الاساط الحيوية المختلفة الى الانواع المختلفة. تم تشخيص ٤٨ عينة من العينات المعزولة التى تم الحصول عليها من الانسان حيث تم عزل ١٥ عينة ميكروسبورم كانز، وعدد ١٢ عينة ترايكوفاييتون فيوليشيم ، وعدد ١٢ عينة ترايكوفاييتونروبرريم ، وعدد ٥ عينة ايدرماتوفيونفلوكوزيموعدد ٤ عينة ترايكوفاييتون منتجروفايت تم تشخيص جميع العينات المعزولة من الحيوان (الماشية) وعددها ثمانية الى ترايكوفاييتون فريكويزيم ، في حين تم تشخيص أربع عينات تم الحصول عليها من الكلاب والقطط ميكروسبورم كانز ومن ناحية أخرى تم تشخيص العينات المعزولة من التربة ميكروسبورمجيبيسيم

ومن الجهة الاخرى استخدمت ثلاث طرق الجينية (الوراثية) لدراسة عدد ٣٠ من العينات المعزولة والتي تم تحديدها سابقا بالطريقة التقليدية (الطريقة المظهرية) والتي تشمل:

(أ) اختبار انزيم البلمرة المتسلسل (PCR) لدراسة عدد ٣٠ من العينات المعزولة لتضخيم ITS4 و ITS1 متبوعة باستخدام انزيم التقطيع MvaI بواسطة (RFLP)

(ب) إختبار انزيم البلمرة المتسلسل لدراسة عدد ٣٠ من العينات المعزولة باستخدام برايمر 4 (GACA) وتم دراستها ايضا باستخدام (RFLP)

(ج) اختبار تسلسل الحمض النووي الذي تم لعدد ٥ عينات وهى ميكروسبورم كانز ، ترايكوفاييتونفريكويزيم ، ترايكوفاييتونفيوليشيموترايكوفاييتونروبرريم

تم تأكيد تطابق التشخيص التقليدى مع التشخيص الوراثة فى جميع الحالات التى تم دراستها ومقارنتها باستخدام (RFLP) مع انزيم التقطيع MvaI وكذلك 4 (GACA). باستثناء حالة ترايكوفاييتون روبررم نسبة تطابقها باستخدام تسلسل الحمض النووي كانت ٩٩٪ وشخصت ترايكوفاييتونراوتشيكي

على الرغم من أن التشخيص الوراثة هو الاسرع ويمثل التقدم التكنولوجي في التشخيص المختبري، إلا أنه مكلف. لذلك نوصى باستخدام التصنيف بالطرق الوراثة فى حالة عدم تواجد متخصص فى تصنيفالفطريات الجلدية او وجود عزلات غير نمطية لا يسهل تصنيفها او فى الدراسات الابدميولوجية.