Valley International Journals
Open Access Journal
New Thinking New Innovation

International Journal of Medical Science and Clinical Inventions
Volume 2 issue 09 2015 page no. 1336-1348 ISSN: 2348-991X
Available Online At: http://valleyinternational.net/index.php/our-jou/ijmsci

Evaluate The Phenotypic And Genotypic Variations For Isolates Of Trichophyton Spp, Isolated From Different Habitats

Kawther Mohammed Ali Hasan[1] ,Majed Kadhum Aboud Al-Shibli[2]
[1]College of Science for Girls, Babylon University, Hilla, Iraq
[2]College of Education, Al-Qadisya University, Al-Qadisya, Iraq
Correspond author: Kawther M.A. Hasan
Email-kawtherali1972@yahoo.com

Abstract
Identification of dermatophytes using conventional methods is unstable because macro- and micromorphological characteristics of these fungi are not clear. Therefore the aim of this study was to evaluate the phenotypic and genotypic variations using specific primer for dermatophytes and Trichophyton genus and sequencing of ITS region. By conventional morphological methods identified 105 isolates of Trichophyton spp. collected from different habitats (76 from human, 17 from animals and 12 from soil). Seventy four (74) isolates of Trichophyton spp. were used for molecular identification by using specific primer pair for dermatophytes had monomorphic bands of approximately PCR product of 366 bp and specific primer pair for Trichophyton genus had monomorphic bands of approximately PCR product of 258 bp. In addition to Trichophyton isolates were sequenced and analyzed by compared the sequence alignment with sequences from NCBI Blast database. These sequences are useful for identification of isolates have not been able to reach species level. Genotypic methods provides a very easy and accurate methods.

Key words: Trichophyton spp., Polymerase chain reaction (PCR), Internal transcribed spacer (ITS), Sequencing.

Introduction

Dermatophytes are a group of molds related morphology and physiology which have the ability to invade keratinocytes tissue in humans and animals, causing superficial cutaneous infections commonly called dermatophytoses (tinea or ringworm) (Simpanya, 2000). Identification of individual dermatophyte species causing infection remains important for several reasons: First, dermatophytosis is often connected with epidemiological circumstances promoting reinfection , as sources of potentially inoculums lead to reinfection in these diseases raise the level of risk. Second, the actual treatment regimens may differ for different dermatophyte species: for example, *Trichophyton tonsurans* in tinea capitis tends to require shorter treatment times than *M. canis*. The latter fungus to some extent evades drug exposure by forming arthroconidia outside the hair shaft, while the former forms arthroconidia inside the hair shaft where contact with conventional antifungal drugs is relatively high (Gräser *et al*., 2008). Third, especially in onychomycosis,it may

DOI: 10.18535/ijmsci/v2i9.09

be needed to culture and species identification because these diseases caused by many types of fungi, some nondermatophytes that does not respond to anti-dermatophyte therapy and also onychomycosis due to dermatophytes is clinically indistinguishable from onychomycosis caused by nondermatophytes (Hilmioglu-Polat *et al*., 2005; Malik *et al*., 2009).

Remains the evolutionary origin of dermatophytes is not clear because the members of this group are evolutionary and taxonomically close and overlapping and relevant with each other as the phenotypic characteristics of diagnostic classification criteria are weak and many clinical isolates are devoid of sexual phase. The processes of speciation in dermatophytes, especially anthropophilic dermatophytes, appear to have been anomalous, and species themselves may be difficult to define. Thus, dermatophytes pose an ongoing problem not only in practical species identification but also in theoretical species conceptualization (Gräser *et al*., 2006; Gräser *et al*., 2008).

Because phenotypic traits of the fungus are unstable, which are characteristic in primary cultures, easily are lost in repeated transfers, this diminishes their value for taxonomy, which necessarily includes the re- examination of old reference strains (Gräser *et al*, 2000). Most of the molecular diagnosis of dermatophytes studies have relied on the study sequence of nitrogenous bases in the internal transcribed spacer regions (ITS) in the rDNA, Erhard *et al*., (2007) used primer pair LR1 and SR6R for sequencing of ITS region in isolates of *T. rubrum* and *T. interdigitale*. While Symoens *et al.*, (2013) used primer pair ITS1 and ITS2 for sequencing of zoophilic and anthropophilic isolates of *T. mentagrophytes* complex, addition to specific primers for dermatophytoses (Makimura *et al*., 1998; Makimura *et al*., 1999; Summerbell *et al*.,1999).

The aim of our study was to evaluate the phenotypic and genotypic variations based on specific primer for dermatophytes and *Trichophyton* genus and used universal primer for sequencing of ITS region of anthropophilic, zoophilic and giophilic isolates of *Trichophyton* spp .

Material and methods

Samples collection and cultural characters

A total of 105 isolates of *Trichophyton* spp. collected from different habitats (76 clinical cases from human, 17 from animals and 12 from soil). Conventional morphological methods were employed for identification of these fungi where dermatophytes were cultured on Sabouraud`s Dextrose agar (SDA) with chloramphenicol and cycloheximide and cultured at 26˚C for up to 4 weeks. The identification of fungal agent was based on macro- and micromorphological characteristics. In addition, fungal identification was confirmed by the in vitro hair perforation test, urease production in Christensen's medium and vitamin requirements in Trichophyton agar media.

DOI: 10.18535/ijmsci/v2i9.09

Extraction of genomic DNA

Seventy four isolates of *Trichophyton* spp. were used for DNA extraction and PCR assay. DNA were extracted by picking 1g of mycelia by using sterile loop and suspending into 300 µl of lyses buffer (10 mM Tris, 1mM EDTA (pH8), 1% SDS, 100mM NaCl, 300 µl phenol-chloroform (1:1)) shaken for 5 min and centrifuged at 1000rpm, the supernatant was transferred to new tube and equal volume of chloroform was added, mixed and centrifuged, the supernatant was transferred to new tube, 500 µl of 70% ethanol alcohol was mixed with supernatant and centrifuged at 10000 rpm for 7 min, dry DNA pellet was re-suspended in 75 µl of TE buffer and stored at -20˚C until use (Al-Khafajii, 2014).

Specific primer –PCR assay

The phenotypic results were confirmed by specific primer pair panDerm1 (5’GAAGAAGATTGTCGTTTGCATCGTCTC 3’) and panDerm2 (5’CTCGAGGTCAAAAGCACGCCAGAG 3’) derived from the chitin synthase 1 (*chs 1*) gene, which is common for all of dermatophytes amplified target 366 bp (Abo El-Yazeed *et al.*, 2013). And specific primer pair Trichopyros-f (5’ GGTGAACTGCGGAAGGATC 3’) and Trichopyros-r (5’ ACGCTCAGACTGACAGCTCTT 3’) derived from rDNA of *Trichophyton* genus amplified target 258 bp (Brillowska-Dabrowska, 2010).

1 µL of DNA (20 µg/ml) from each of isolates were mixed with PCR mixture (final reaction volume 25 µL) consisted of 12 µL of 2x Master Mix (Promega), 2 µL of primers (10 pmole) and rest molecular-grade water. The PCR condition for panDerm1 and panDerm2 primers were initial denaturation at 95˚C for 7 min followed by 40 cycles denaturation at 94˚C for 40 sec, annealing at 60˚C for 45 sec and extension at 72˚C for 1 min followed by final extension at 72˚C for 7 min. The PCR condition for Trichopyros-f and Trichopyros-r primers were initial denaturation at 94˚C for 5 min followed by 40 cycles denaturation at 94˚C for 30 sec, annealing at 50˚C for 30 sec and extension at 72˚C for 30 sec followed by final extension at 72˚C for 5 min. The PCR mixture was amplified by thermal cycler PCR System (Labnet, USA).

The PCR products for each target region were run on 1.2% agarose gel (Bio Basic Canada Inc.). Electrophoresis was performed at 100 V. in TBE buffer. The gel was pre-stained with 0.05% ethidium bromide. The DNA bands were detected by Desktop Gel imager scope 21 ultraviolet transilluminator (Korea Com.).

Sequencing assay

To study the relationship and similarity at morphological and molecular level that are sometimes exhibited among *Trichophyton* spp., the genomic DNA of the representative isolates for *Trichophyton* spp were amplified with universal primers ITS5 and ITS4 (Brilhante *et al.*,

DOI: 10.18535/ijmsci/v2i9.09

2006). After PCR amplification, the purified products for *Trichophyton* isolates were sequenced by sent the PCR products to the Macrogene Company, USA. The sequence alignment was compared with sequences from NCBI Blast database to get the highest percentage of match in the name of genus and species for sexual and asexual phase of each isolate. For the purpose of reaching a final diagnosis of the isolates were compared with the findings of the study in the phenotypic diagnosis of isolates under study with the findings of the molecular diagnostics it.

Results and Discussion

Phenotypic identification

A total of 105 isolates of *Trichophyton* spp. collected from different habitats (76 clinical cases from human, 17 from animals and 12 from soil). According to the macro- and micromorphological characteristics, physiologic and biochemical test were identified nine species of *Trichophyton* genus. In addition to the number of isolates scrupulous diagnosed as belonging to *Trichophyton* genus, we have unable to diagnosis at species level because they lost to cultural characters like clear colony and micro- or macroconidia (Table 1).

Table (1): *Trichophyton* species and their number and percentage isolated from different habitats.

species	human	animal	soil	total No.	%
T. equinium	3	-	-	3	2.85
T. interdigetale	16 (21.05)	-	2	18	17.14
T. mentagrophytes	13 (17.10)	7	5	25	23.80
T. rubrum	7	-	-	7	6.66
T. schoenleinii	2	-	-	2	1.90
T. soudanense	2	-	-	2	1.90
T. tonsurans	2	-	-	2	1.90
T. verrucosum	9	7	-	16	15.23
T. violaceum	7	-	-	7	6.66
Trichophyton spp.	15	3	5	23	21.90
Total No.	76	17	12	105	100

DOI: 10.18535/ijmsci/v2i9.09

The results showed that *Trichophyton* species isolated from clinical cases of human, *T. interdigetale* (16/76) have occupied the first place either *T. mentagrophytes* (13/76) came in second place, *T. verrucosum* (9/76) came third place, the lowest species were returning to *T. schoenleinii*, *T. soudanense* and *T. tonsurans* (2/76). These results were consistent with the results of Abastabar *et al*. (2013) that *T. interdigetale* have occupied the highest frequency rate (48.75%) while García-Martos *et al*. (2004) that *T. mentagrophytes* have occupied the highest frequency rate (24.87%). The reason for the difference may be due to the local patterns of fungal isolates from clinical cases may vary depending on the time and geographical location so it is important to isolate local species and diagnosis constantly (Prasad *et al*., 2013).

Genotypic identification

It was selected 74 isolation scrupulous diagnosed as belonging to *Trichophyton* genus, some of them have been identified to the species level and others were not identified at species level. These isolates were selected to representative different habitats by 55 isolates from human numbered 1-55 and ten isolates from animals numbered 56-65 and nine isolates from soil numbered 66-74 of gel electrophoresis (figures 1 & 2) to primers used in molecular diagnostics.

The PCR products were obtained by specific primer pair panDerm1 and panDerm2 amplified target 366 bp (Figure 1), This primer has succeeded in typing 90.54% (67/74) of the isolates under study, the except isolates numbered 29, 36, 37, 42 and 44 isolated from human and numbered 72 and 73 isolated from soil. There are many studies that used the same primer, Brillowska-Dabrowska *et al.* (2007) able to typing dermatophytes from non-dermatophytes of clinical cases infected with onychomycosis. As happened Abo El-Yazeed *et al*. (2013) on 22 isolates (73.3%) isolated from clinical cases of horses while Garg *et al*. (2007) happened on the proportion of 79.6% using the nested PCR from clinical samples of onychomycosis. As used Dabrowska *et al*. (2014) in typing 7 isolates belonging to *T. mentagrophytes* complex and 8 isolates of *M. canis* isolated from cats and dogs using three different annealing temperatures 54˚C, 56˚C and 58˚C The third degree is the best.

DOI: 10.18535/ijmsci/v2i9.09

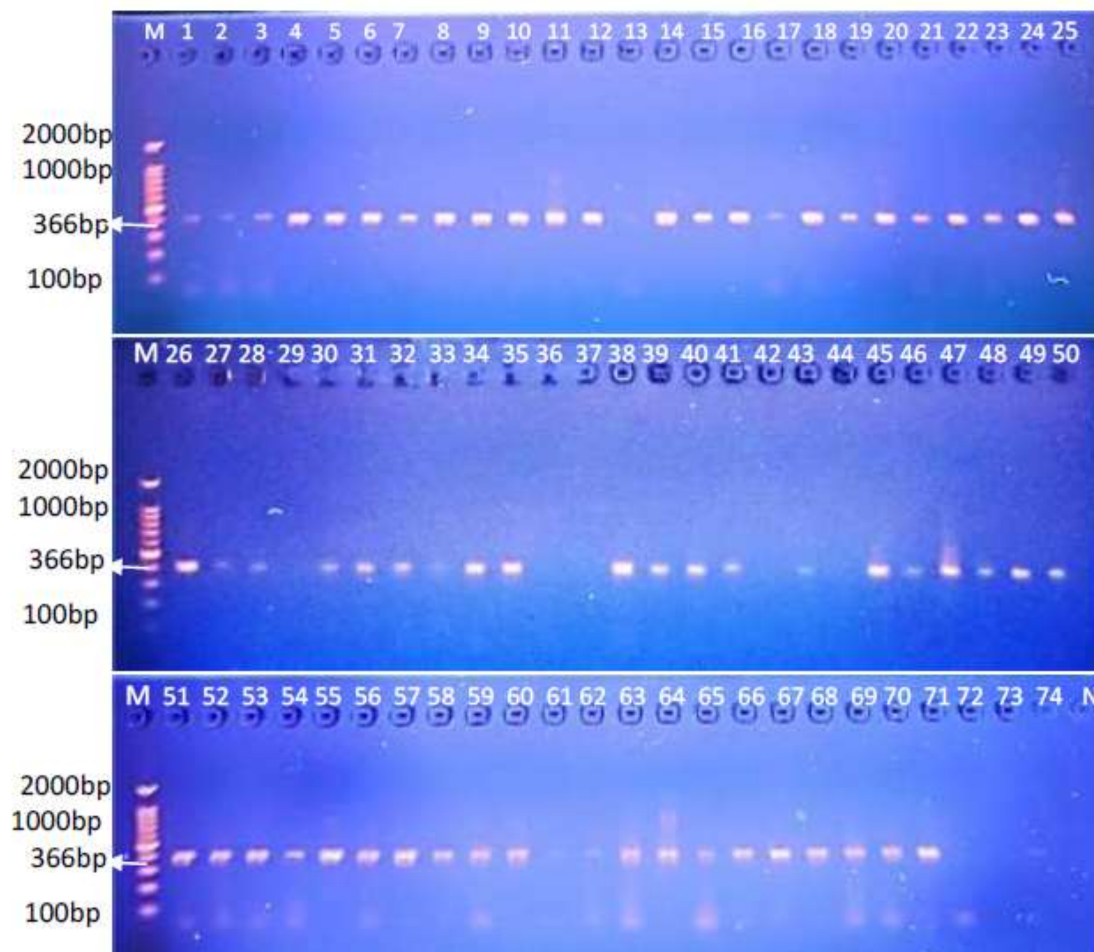

Figure (1): Electrophoretic patterns of PCR products with pan-Derm primers to 74 isolates of *Trichophyton* spp. numbered 1-55 from human, 56-65 from animals and 66-74 from soil, M= Ladder 100bp, N= Negative control.

The PCR products were obtained by specific primer pair Trichopyros- f and Trichopyros-r amplified target 258 bp (Figure 2), This primer has succeeded in patterning 95.94% (71/74) of the isolates under study, the except isolate numbered 6 isolated from human and numbered 64 and 65 isolated from animals although these isolates were typing by panDerm1 & panDerm2 primer, the reason may be that these isolates are not of *Trichophyton* genus or the primer failed to typing these isolates. Brillowska-Dabrowska (2010) was reported that among the 23 sample of dermatophytes was 21 a sample is positive for the Trichopyros primers and pander primers either two samples remaining, one was positive for the first primer and negative for the second primer and the other opposite, and pointed

DOI: 10.18535/ijmsci/v2i9.09

out that there is very little error ratio when diagnosing dermatophytes of these two primers.

Figure (1): Electrophoretic patterns of PCR products with Trichopyros primers to 74 isolates of *Trichophyton* spp. numbered 1-55 from human, 56-65 from animals and 66-74 from soil, M= Ladder 100bp, N= Negative control.

For the sequencing of these isolates by using purified products of universal primers ITS5 and ITS4. Table 2 shows the comparison of the results of the phenotypic diagnosis isolates of *Trichophyton* spp. under study with the results of its molecular diagnostics (Table 2), noted that molecular diagnostics using the sequence of nitrogenous bases for most of the samples were identical to the phenotypic diagnosis using traditional methods with the exception of isolate numbered 6 which was diagnosed by conventional methods as *T. mentagrophytes*, but the results of molecular diagnostics as type *A. otae* , this may explain it the lack of response to Trichopyros-f & Trichopyros-r primers, and isolates numbered 8,11 and 12 which were diagnosed as *T. quinckeanum*, sources confirm that this species is one of the variety of *T. mentagrophytes* (Beguin *et al.*, 2012).

DOI: 10.18535/ijmsci/v2i9.09

As possible diagnosis of isolates that we did not reach its species by using traditional methods that isolates numbered 41-55 from human, 63-65 from animals and 70-74 from soil, these results were consistent with the results of a number of sources, Li *et al*. (2008) identified 126 isolates by using the sequence of nitrogenous bases of three different region ITS1, ITS2 and D1-D2 in order to get the highest match and compare it with the phenotypic diagnosis. While Bergmans *et al.* (2008) used two molecular methods for dermatophytes identified first PCR–reverse line blot (PCR-RLB) method and second the sequencing of ITS1 region. Where confirmed Brasch & Graser (2005) that *Chrysosporium* and sexual phase *A. multifidum* have 98% percent match with the species of *Trichophyton*.

Table (2): the comparison of the phenotypic diagnosis isolates of *Trichophyton* spp. its molecular diagnostics

No. isolate	**Phenotypic identification**	**Molecular identification**		**Final identification**
		ITS/bp	**Sequencing %**	
1-3	*T. equinum*	720	*T. equinum* 98% *T. mentagrophytes* 98%	*T. equinum*
4-5	*T. mentagrophytes*	720	*T. mentagrophytes* 99% *A. benhamiae* 99%	*T. mentagrophytes*
6	*T. mentagrophytes*	780	*A. otae* 95%	*A. otae*
7	*T. mentagrophytes*	680	*T. interdigetale* 95% *A. vanbreuseghemii*95%	*T. interdigetale*
8	*T. mentagrophytes*	720	*T. quinckeanum* 98% *T. mentagrophytes* 97%	*T. mentagrophytes* var. *quinckeanum*
9-10	*T. mentagrophytes*	720	*T. mentagrophytes* 99% *A. benhamiae* 99%	*T. mentagrophytes*
11-12	*T. mentagrophytes*	720	*T. quinckeanum* 98% *T. mentagrophytes* 97%	*T. mentagrophytes* var. *quinckeanum*
13, 19, 22	*T. interdigetale*	720-800	*T. interdigetale* 99% *A. vanbreuseghemii*99%	*T. interdigetale*
14,18, 21	*T. interdigetale*	720-780	*T. interdigetale* 100% *T. verrucosum* 98%	*T. interdigetale*

DOI: 10.18535/ijmsci/v2i9.09

15-16	*T. interdigetale*	780	*T. interdigetale* 99% *T. mentagrophytes* 99%	*T. interdigetale*
17	*T. interdigetale*	780	*T. interdigetale* 99% *T. verrucosum* 98%	*T. interdigetale*
20	*T. soudanense*	780	ND	*T. soudanense*
23	*T. rubrum*	680	*T. rubrum* 100%	*T. rubrum*
24	*T. rubrum*	800	*T. rubrum* 98% *T. interdigetale* 96%	*T. rubrum*
25-27	*T. rubrum*	780- 800	ND	*T. rubrum*
28	*T. tonsurans*	780	*T. tonsurans* 85% *T. interdigetale* 84%	*T. tonsurans*
29	*T.schoenleinii*	780	ND	*T.schoenleinii*
30	*T. verrucosum*	780	*T. verrucosum* 100%	*T. verrucosum*
31-32	*T. verrucosum*	720- 780	*T. verrucosum* 99% *T. interdigetale* 97%	*T. verrucosum*
33-34	*T. verrucosum*	780	*T. verrucosum* 99% *T. interdigetale* 99%	*T. verrucosum*
35	*T. verrucosum*	720	*T. verrucosum* 99% *T. interdigetale* 98%	*T. verrucosum*
36-37	*T. violaceum*	720	ND	*T. violaceum*
38-39	*T. violaceum*	720	*T. violaceum* 96% *T. soudanense* 95%	*T. violaceum*
40	*T. violaceum*	720	*T. violaceum* 97% *T. rubrum* 95%	*T. violaceum*
41	*Trichophyton* sp.	780	*T. interdigetale* 99% *A. vanbreuseghemii*99%	
42-44	*Trichophyton* sp.	780	*A. otae* 90-95%	*A. otae*
45-46, 49-50, 52, 54	*Trichophyton* sp.	700- 720	*T. interdigetale* 99% *T. mentagrophytes* 99%	*T. mentagrophytes* complex
47	*Trichophyton* sp.	720	*T. interdigetale* 99%	*T. interdigetale*

DOI: 10.18535/ijmsci/v2i9.09

			*A. vanbreuseghemii*99%	
48	*Trichophyton* sp.	720	*T. verrucosum* 99% *A. vanbreuseghemii*99%	*T. verrucosum*
51, 53	*Trichophyton* sp.	720	*T. verrucosum* 100%	*T. verrucosum*
55	*Trichophyton* sp.	700	*T. mentagrophytes* 99% *A. benhamiae* 99%	*T. mentagrophytes*
56-57	*T. mentagrophytes*	720	*T. mentagrophytes* 99% *A. benhamiae* 99-100%	*T. mentagrophytes*
58	*T. mentagrophytes*	720	ND	*T. mentagrophytes*
59-60	*T. verrucosum*	680-720	ND	*T. verrucosum*
61-62	*T. verrucosum*	720-780	*T. verrucosum* 100%	*T. verrucosum*
63	*Trichophyton* sp.	780	*T. interdigetale* 99% *T. mentagrophytes* 99%	*T. mentagrophytes* complex
64	*Trichophyton* sp.	720	*T. verrucosum* 100%	*T. verrucosum*
65	*Trichophyton* sp.	720	*T. verrucosum* 99% *A. vanbreuseghemii*99%	*T. verrucosum*
66	*T. mentagrophytes*	700	ND	*T. mentagrophytes*
67	*T. mentagrophytes*	700	*T. mentagrophytes* 99% *A. benhamiae* 99%	*T. mentagrophytes*
68	*T. interdigetale*	700	ND	*T. interdigetale*
69	*T. interdigetale*	780	*T. interdigetale* 99% *A. vanbreuseghemii*99%	*T. interdigetale*
70, 74	*Trichophyton* sp.	780	*T. interdigetale* 99% *T. mentagrophytes* 99%	*T. mentagrophytes* complex
71	*Trichophyton* sp.	620	*A. multifidum* 99%	*Chrysosporium*
72-73	*Trichophyton* sp.	620-680	*Ch. tropicum* 99%	*Ch. tropicum*

ND= Not determined

References

Abastabar, M.; Rezaei-Matehkolaei, A.; Shidfar, M.R. & *et al*., (2013). A molecular

DOI: 10.18535/ijmsci/v2i9.09

epidemiological survey of clinically important dermatophytes in Iran based on specific RFLP profiles of beta tubulin gene. Iranian J Publ Health, 42(9):1049-1057.

Abo El-Yazeed, H.; Effat, M.; Abdalla, K.; Bakry, M.; Alarousy, R. & Farahat, E. (2013). Application of molecular techniques for rapid diagnosis of dermatophytes infection in horses. Global Veterinaria 10 (3): 310-317.

Al-khafajii, K. (2014). Myco- epidemiologic and genetic of dermatophytosis and non-dermatophytes in Middle Euphrate, Iraq. African J. Microbiolo. Research, 8(24):2381-2386.

Beguin, H.; Pyck, N.; Hendrickx, M.; Planard, C.; Stubbe, D. & Detandt, M. (2012). The taxonomic status of *Trichophyton quinckeanum* and *T. interdigitale* revisited: a multigene phylogenetic approach. Med. Mycol., 50:871–882.

Bergmans, A.M.C.; Schouls, L.M.; van der Ent, M.; Klaassen, A.; Bohm, N.; & Wintermans, R.G.F. (2008). Validation of PCR–reverse line blot, a method for rapid detection and identification of nine dermatophyte species in nail, skin and hair samples. Clin. Microbiol. Infect., 14: 778–788.

Brilhante, R.S.N.; Cordeiro, R.A.; Gomes, J.M.F.; Sidrim, J.J.C. & Rocha, M.F.G. (2006). Canine dermatophytosis caused by an anthropophilic species: molecular and phenotypical characterization of *Trichophyton tonsurans*. Journal of Medical Microbiology 55:1583–1586.

Brillowska-Dabrowska, A.H. (2010). PCR diagnostics of dermatophytes and other pathogenic fungi. European patent specification, :1-11.

Brillowska-Dabrowska, A.; Saunte, D.M. & Arendrup, M.C. (2007). Five-hour diagnosis of dermatophyte nail infections with specific detection of *Trichophyton rubrum*. J. Clin. Microbio., 45(4):1200-1204.

Brasch, J. & Graser, Y. (2005). *Trichophyton eboreum* sp. nov. isolated from human skin. J. Clin. Microbiology, 43(10):5230–5237.

Dabrowska, I.; Dworecka-Kaszak, B. & Brillowska-Dabrowska, A. (2014). The use of a one-step PCR method for the identification of *Microsporum canis* and *Trichophyton mentagrophytes* infection of Pets. Acta Biochimica Polonica, 61(2):375-378.

Erhard, M.; Hipler, U.; Burmester, A.; Brakhage, A.A. & Wostemeyer, J. (2007). Identification of dermatophyte species causing onychomycosis and tinea pedis by MALDI-TOF mass spectrometry. J. compilation Experimental Dermatology p:1-6.

García-Martos, P.; Ruiz-Aragón, J.; García-Agudo, L. & Linares, M. (2004). Dermatophytoses due to *Microsporum gypseum*:

DOI: 10.18535/ijmsci/v2i9.09

Report of eight cases and literature review. Rev Iberoam Micol; 21: 147-149.

Garg, J.; Tilak, R.; Singh, S.;Kumar, G.; Garg, A.; Prakash, P. & Nath, G. (2007). Evaluation of pan-dermatophyte nested PCR in diagnosis of onychomycosis. J. Clin. Microbio., 45(10):3443-3445.

Gräser, Y.; de Hoog, G.C. & Kuijpers, A.F.A. (2000). Recent advances in the molecular taxonomy of dermatophytes. In: Biology of dermatophytes and other keratinophilic fungi. Revista Iberoamericana de Micologia Bibao (Spain) :17-21.

Gräser, Y.; de Hoog, G.S. & Summerbell, R. (2006). Dermatophytes:
recognizing species of clonal fungi. Med Mycol. 44:199–209.

Gräser, Y.; Scott, J. & Summerbell, R.(2008). The new species concept in dermatophytes - a Polyphasic approach. Mycopathologia 1-18.

Hilmioglu-Polat, S.; Metin, D.Y.; Inci, R.; Dereli, T.; Kilinc, I. & Tumbay, E. (2005). Non-dermatophytic molds as agents of onychomycosis in Izmir, Turkey – a prospective study. Mycopathologia 160: 125–128.

Li, H.C.; Bouchara, J.; Hsu, M.M.; Barton, R.; Su, S. & Chang, T.C. (2008). Identification of dermatophytes by sequence analysis of the rRNA gene internal transcribed spacer regions. J. Med. Microbio. 57: 592–600.

Makimura,K.; Mochizuki, A.; Hasegawa, A.; Uchida, K.; Saito, H. & Yamaguchi,H. (1998). Phylogenetic classification of *Trichophyton mentagrophytes* complex strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J. Clin. Microbiol. 36(9):2629.

Makimura,K.; Tamura, Y.; Mochizuki, A.; Hasegawa, A.; Tajiri, Y.; Hanazawa, R.; Uchida, K.; Saito, H. & Yamaguchi, H. (1999). Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences of nuclear ribosomal internal transcribed Spacer 1 regions. J. Clin. Microbiol. 37(4):920-924.

Malik, N.A.; Raza, N.& Nasiruddin (2009). Non-dermatophyte moulds and yeasts as causative agents in onychomycosis. Journal of Pakistan Association of Dermatologists, 19: 74-78.

Prasad, N.; Mahapatra, A. & Chayani, N. (2013). Changing trends in the fungal isolates from clinical specimens of suspected superficial mycosis. Indian Medical Gazette, 60-62.

Simpanya, M.F. (2000). Dermatophytes: Their taxonomy, ecology and pathogenicity .In:Biology of dermatophytes and other keratinophilic fungi. Revista Iberoamericana de Micologia Bibao (Spain) :1-12.

DOI: 10.18535/ijmsci/v2i9.09

Summerbell, R. C.; Haugland, R. A.; Li, A. & Gupta, A. K. (1999). rRNA Gene internal transcribed spacer 1 and 2 sequences of asexual, anthropophilic dermatophytes related to *Trichophyton rubrum*. J. Clin. Microbiol. 37(12):4005-4011.

Symoens, F.; Jousson, O.; Packeu, A.; Fratti, M.; Staib, P.; Mignon, B & Monod, M. (2013). The dermatophyte species *Arthroderma benhamiae*: intraspecies variability and mating behavior. Journal of Medical Microbiology (2013), 62, 377–385.

DOI: 10.18535/ijmsci/v2i9.09