Journal of University of Babylon, Pure and Applied Sciences,Vol.(26), No.(5): 2018

Evaluation The Phenotypic and Genotypic Variations for Clinical Isolates of *Arthroderma otae* Complex

Kawther Mohammed Ali Hasan[1]

[1]*College of Science for Women, Babylon University, Hilla, Iraq.*

E-mail: kawtherali1972@yahoo.com

Abstract

M. canis , *M. audouinii* and *M. ferrugineum* are three species of*Arthroderma otae* com- plex, which morphologically and genetically closely related. Conventional identification of phenotypic and biochemical characteristics are usually yielding unclear results. Geno- typic identification by using modern technique which is yielding more accurate results. In this study, ITS region of rDNA was sequenced and phylogenetic tree analysis for *Mi- crosporum* spp. were observed three clusters of *A. otae* isolates. When sequencing identi- fies with the reference strains sequence of GenBank Database detected several mutations in multiple locus either by miss matching or genetic gaps. By using Mega 6 software and display toggle conserved sites at the 50% level with different colors of *A. otae* isolates showed identification of the colors in the nitrogenous bases alignment belong to the same species of the telomorph.

Keywords: *Arthroderma otae* complex, Polymerase chain reaction (PCR), ITS re- gion, Sequencing.

تقييم التغايرات المظهرية والوراثية للعزلات السريرية لمعقد الفطر *Arthroderma otae*

كوثر محمد علي حسن

كلية العلوم للبنات/ جامعة بابل/ حلة – العراق

الخلاصة

الفطريات *M. canis* و*M. audouinii* و*M. ferrugineum* ثلاثة انواع فطرية تعود لمعقد النوع *Arthroderma otae* وتكون متقاربة جدا مظهريا ووراثيا. التشخيص التقليدي والذي يتضمن كل من الصفات المظهرية والكيميائية الحياتية عادة ما تنتج نتائج غير واضحة مقارنة مع التشخيص الوراثي بواسطة استخدام تقنيات جزيئية حديثة تعطي نتائج اكثر وضوحا. تضمنت الدراسة الحالية اجراء تتابع للقواعد الناتروجينية لمنطقة ITS region في الدنا الرايبوسومي (rDNA) لعدد من عزلات الفطر *Microsporum* spp وتحليل الشجرة التطورية لهذه المنطقة وقد اظهرت النتائج ثلاث مجاميع عنقودية لعزلات الفطر *A. otae* وهو الطور الجنسي للفطر *Microsporum* spp. تمت مطابقة تتابع العزلات قيد الدراسة مع تتابع العينات المرجعية لقاعدة البيانات في البنك الجيني (GenBank Database) وقد اظهرت النتائج عدد من مناطق التطفير متمثلة اما بفقدان التواصل (Miss matching) او الفجوات الوراثية (Genetic gaps). وبواسطة استخدام برنامج Mega 6 software وعرض المناطق المحافظة بالوان مختلفة لكل قاعدة نايترجينية تحت مستوى 50% لعزلات الفطر *A. otae* فقد اظهرت

نتائج التطابق في الوان القواعد النايتروجينية ان جميع العزلات تنتمي الى نفس النوع في الطور الجنسي.

الكلمات المفتاحية: مجمع أرثروديرما أوتاي، تفاعل البوليميراز المتسلسل (بر)، إيتس ريجيون، التسلسل.

1-Introduction

Arthroderma otae complex includes three species of *Microsporum*, which morphologi- cally and genetically closely related are the zoophilic *M. canis* and the anthropophilic *M. audouinii* and *M. ferrugineum*. Although they ecologically are different, but they all cause human infections are clinically similar, so the distinction between these species of important epidemiologically [1] [2]. The most common species globally in this complex is *M. canis*. This species has two varieties, *M. canis* var. *canis* which producing beaked rough walled macroconidia with low numbers of microconidia and *M. canis* var. *distortum* which producing distorted macroconidia with large numbers of microconidia. The second member of these complex is *M. audouinii*, it is closest to the *M. canis* of morphologic and microscopic characteris- tics, some isolates produce rough walled beaked macroconidia with medial constriction and others produce only microconidia. The third rare member is *M. ferrugineum*, its colonies similar to *M. canis* but do not be conidia with strongly septate hyphae (bamboo hyphae) [3].

Because most phenotypic characteristics of the dermatophyte species as to be almost in similar that these qualities are often lost when repeated transfers, so the modern classi- fication do not very much depends on phenotypic traits and it has replaced the molecular diagnostics. Molecular identification studies of the dermatophyte species by microsatel- lite primers for detection of *M. audouinii* [4], specific primers for *M. canis* and *Trichophyton tonsurans* [5], determination of the base sequence of ITS region and D1-D2 region [6], and restriction frag- ment length polymorphism (RFLP) for identification of dermatophyte species [7] [8] [9].

But the species that belong to the same complex need to be more accurate techniques than traditional molecular methods in order to know the slight genetic differences between these species. Several studies used specific primers for dermatophytes strains and other fungi and phylogenetic classification by several regions of rDNA sequences [10] [11]. The specific rDNA sequences of ITS2 region in species of *M. canis* complex was determined and analyzed phylogenetically by [12]. Detection and identification of dermatophytes by multiplex real- time PCR [13]. [14] were detected mycovirus in the fungi which include dermatophyte and non- dermatophyte by molecular methods.

The purpose of this search was to estimate the phenotypic and genotypic variations in clinical isolates of *Microsporum* spp. belong to *Arthroderma otae* complex by using traditional and molecular methods.

2-Material and methods

Fungal isolates and Conventional identification: An overall of 50 clinical fungal isolates of *Microsporum* spp. were taken from patients with dermatophytoses from the Unit of Mycology in Hilla Hospital (Iraq). All isolates included (60%) from male and (40%) from a female who clinically diagnosed by dermatologist in Hospital. Fungal strains were cultured on Sabouraud's medium (SDA) with antibacterial chloramphenicol and antifungal cycloheximide at 26°C for up to 4 weeks.The diagnoses of fungal isolates was based on characteristics of macro- and micromorphology. In addition, fungal identification was confirmed by physiologic and biochemical properties such as the urease production in Christensen's medium, growth on rice grains, in vitro hair perforation test, and vitamin requirements in Trichophyton agar media according to [15].

3-DNA extraction

Genomic DNA of thirteen isolates of *Microsporium* spp. was extracted and PCR assayed according to [16].

4-PCR assay

The phenotypic results were confirmed by universal primers forward ITS5 (5'AAG-TAAAAGTCGTAACAAGGTTTCCG'3) and reverse ITS4 (5'TCCTCCGCTTATTGATATGC'3) for ITS1-5.8S-ITS2 region of rDNA. PCR mixture was mixed with1 μl of DNA (20 μg/ml) from each of isolates (final reaction volume 25 μl) according to [17] [18]. The PCR mixture was amplified by using thermal cycler system (Labnet, USA).

The electrophoresis for amplification products was carry on 1.2% agarose gel (Bio Basic Canada Inc.) with 0.05% ethidium bromide and performed at 100 V. in TBE buffer and detected of DNA bands by Desktop Gel imager scope 21 ultraviolet transilluminator (Korea Com.).

5-Sequencing assay

To study the similarity and relationship at phenotypic and molecular level among *Mi- crosporum* spp., the purified PCR products for *Microsporum* isolates were sequenced by sending to the Microgene Company, USA. The sequence alignment was compared with sequences from NCBI Blast (GenBank) database to get the highest percentage of the 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 the findings of the phenotypic diagnosis of isolates under study with the findings of the molecular diagnostics it.

6-Phylogenetic analysis

In order to reach the degree of genetic convergence among these isolates, multiple ITS nitrogenous bases sequence alignment were performed by using the MEGA6 software program. Phylogenetic tree analysis applied by using an unweighted pair group method with arithmetic mean (UPGMA) software [19].

7- Results

Conventional identification

Fungal isolates: Three species of *Microsporum* were isolated from clinical specimens on SDA with chloramphenicol and Cycloheximide (Table 1). Twenty nine isolates of *M. canis*, 17 *M. audouinii* and 4 *M. ferrugineum* were identified by morphology and biochemical methods.

Microsporum spp.	tinea corporis	tinea capitis	tinea faciei	Total No.(%)
M. canis	20	7	2	29 (58)
M. audouinii	13	4	0	17 (34)
M. ferrugineum	3	1	0	4 (8)
Total No. (%)	36 (72)	12 (24)	2 (4)	50

Table 1: Shows No. of *Microsporum* spp. isolated from clinical specimens.

8- Macroscopic and microscopic characteristics

In general *M. canis* has white to cream or lemon to yellow colony pigmentation on Sabouraud's dextrose agar, within 14 days producing colonies (7 cm) were flat, dense cotton, fluffy to hairy surface, with yellow to brownish yellow reverse pigment (Fig. 1). In microscopic characteristic, this species has macroconidia with thick-rough-walled, fusoid with 6-12 cells and terminal knob (beaked). Microconidia were unicellular and pyriform-shaped. Racquet hyphae and chlamydospores might be present (Fig. 2).

M. audouinii withen 14 days producing colonies (5 cm) were flat, light ,fluffy surface with some radial grooves, white to peach colored with brown to pink reverse pigment. Microscopically, this species may produce rough-walled, beaked macroconidia with medial constriction or irregular macroconidia, many isolates remain non-sporulating or produce only microconidia, many isolates produce chlamydospores, racquet or pectinate hyphae (Fig.3).

M. ferrugineum withen 14 days producing colonies (3-5 cm) were glabrous to waxy in texture, flat surface and folded at the centre, with white to cream colored and without reverse pigment. Microscopically, this species produced bamboo-like and racquet hyphae, no macro- and micro-conidia produced (Fig. 4).

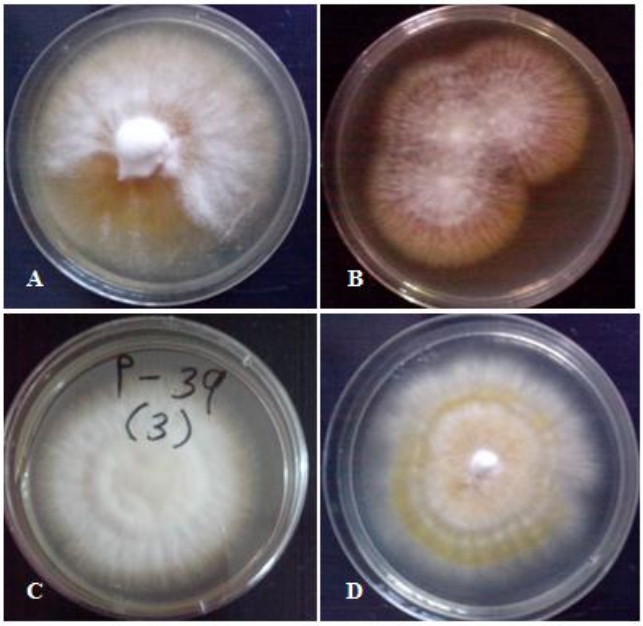


FIGURE1. Different isolates of *M. canis* (A-D) colony morphology on SDA at 28˚C

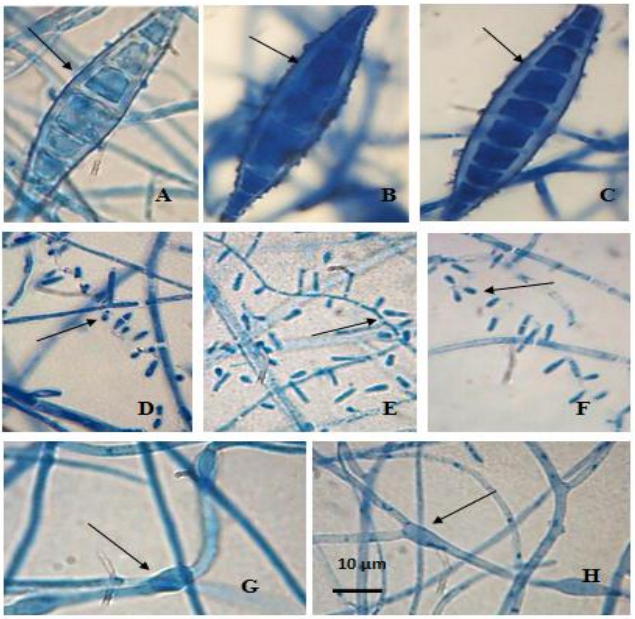


FIGURE 2. Microscopic morphology of *M. canis* isolates.(A-C) Macroconidia from different isolates. (D-F.) Microconidia from different isolates. (G, H) Racquet hyphae.

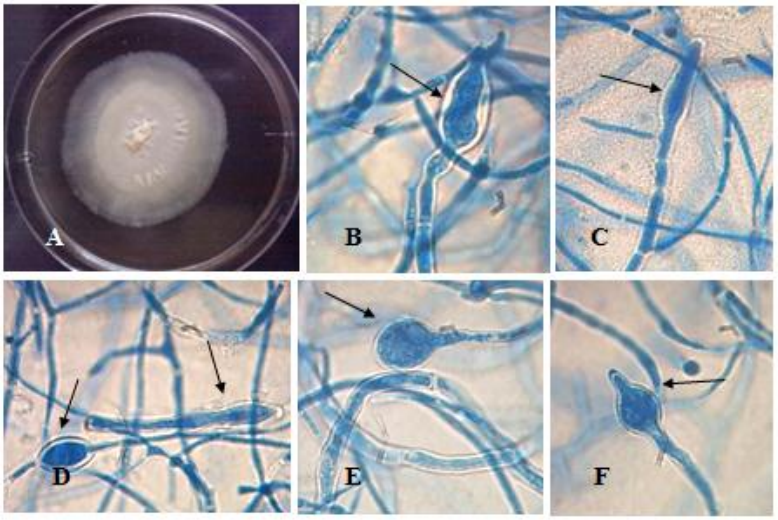


FIGURE 3. (A) Colony of *M. audouinii* . (B-D) Irregular macroconidia with medial constriction. (D) Intercalary chlamedospore. (E,F) Terminal chlamedospore.

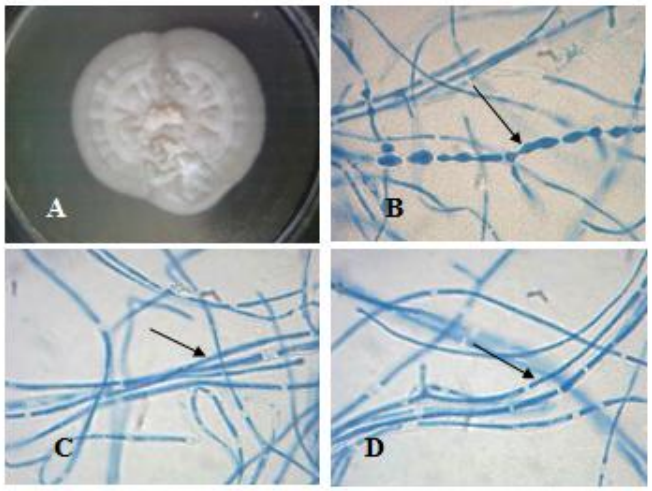


FIGURE 4. (A) Colony of *M. ferrugineum*. (B) Racquet hyphae. (C,D) Bamboo-like hyphae.

9-Molecular identification

Sequencing assay:All isolates of *Microsporum* species were tested at a molecular level to confirm the identification of *Microsporum* species belong to *A. otae* complex. By using conventional PCR technique with primer pairs ITS5 and ITS4 for ITS1, 5.8S and ITS2 regions of rDNA. In gel electrophoresis, the amplicons of ITS region for *A. otae* strains were around 780- 820 bp. Satisfactory sequencing results were obtained, the isolates had been identified by comparison, these isolates sequencing with sequences from NCBI Blast database, all isolates of *Microsporum* species showed a homological percentage from 97% to 100% with *A. otae*. Our results of the sequencing analysis for *Microsporum* isolates was obtained depending on the bases of their ITS region sequences by using the MEGA6 software program. The results of the phylogenetic tree analysis for thirteen isolates of *Microsporum* spp. were observed three clusters of *A. otae* isolates (fig. 5). Phylogenetic tree was showing a high sequence similarity in the ITS region of rDNA between 99.9- 100% similarities for the isolates belong to the cluster 1, but this group with cluster 2 and 3 showed the similarity of about 99.37%.

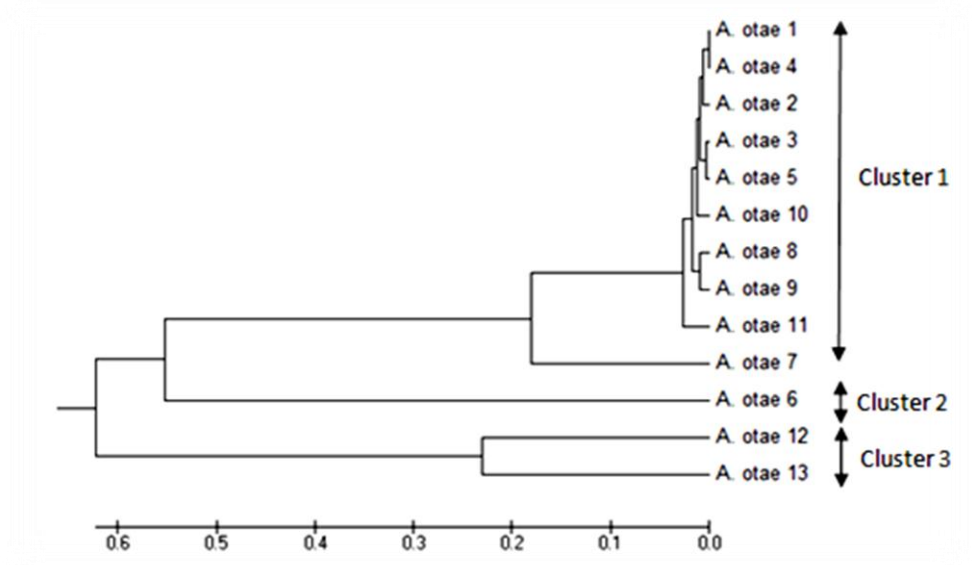
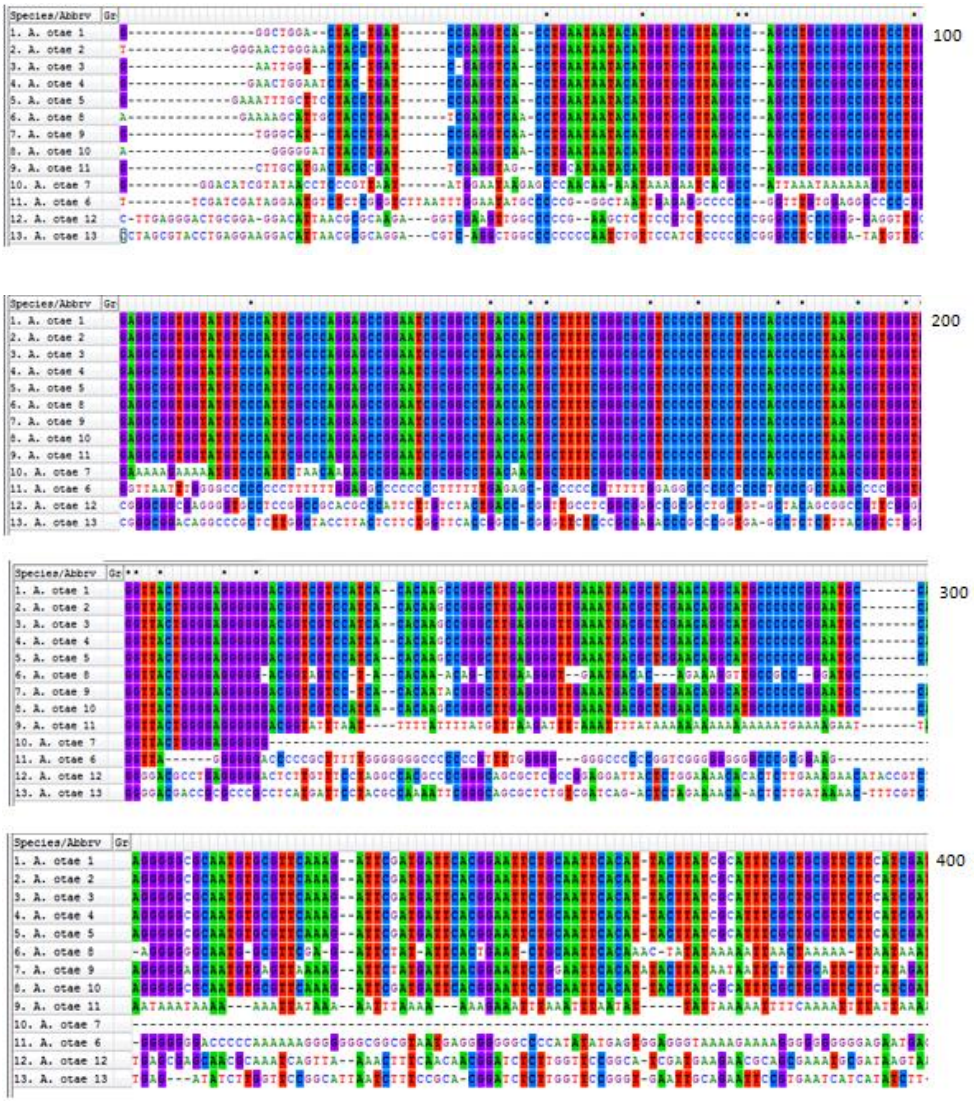


FIGURE 5. Thirteen *Arthroderma otae* strains phylogenetic tree, based on ITS1, ITS2 and 5.8S region rDNA sequences

Sequencing analysis data for thirteen *A. otae* isolates were employed by using Mega 6 software and display toggle conserved sites at 50% level with different colors depending on type of nitrogenous base (T= orange, A= light green, C= blue and G= purple). In this sequencing analysis shows that the identification of the colors in the nitrogenous bases alignment belong to the same species of the telomorph, especially at the 50% level gives a clear view of the convergence or divergence extent of strains from each other. Sequences of all cluster 1 (strains 1-5 and 8-11 of clinical isolates) were approximately similar of sequencing because they have the same color in all isolates. While cluster 2 (strain 6) and cluster 3 (strains 12 and 13) are differences from together in often nitrogenous bases sequencing (fig. 6).



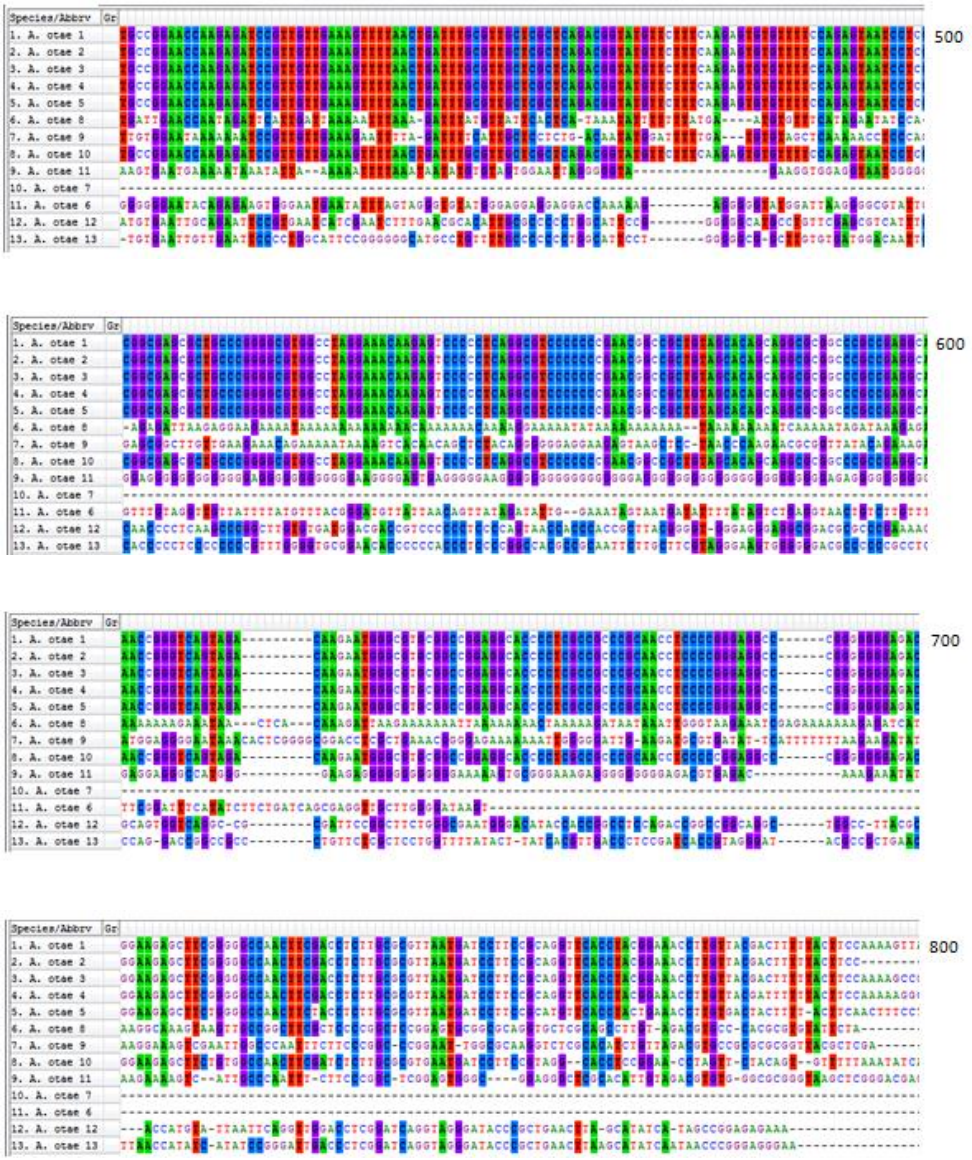


FIGURE 6: sequence data for thirteen *A. otae* isolates by using Mega 6 software, the star of up is show similarity of nitrogen base of all isolates. Different colors are show similarity of nitrogen bases a level of 50%. Number of nucleotide sequence is shown on the right.

Results of table (2) showed *Microsporum* spp. identification and determined by phenotypic methods and genotypic methods by using ITS region analysis and identity with the reference strains sequence of GenBank Database. The isolates of *A. otae* complex (*M. canis*, *M. audouinii* and *M. ferrugineum*) were easily differentiated from each other by ITS region sequence analysis. There was no genetic variation in ITS region of rDNA within *M. canis* which belongs to strains 1-4 and these isolates gave a sequence with 100% identifying to the *A. otae* with closest sequence match of GenBank accession no. JX122197.1. While the remaining of the clinical isolates (5 and 8-11 were *M. canis*, 6-7 were *M. audouinii* and 12 was *M. ferrugineum*) gave the match with 90-99% identity to

the *A. otae* with the exception of strain no. 13 was *M. ferrugineum* that gave the match with 75% identifying to the *Arethroderma* sp. (Table, 2).

Isolates no.	Species identification		Base pair (%) identity with GenBank sequence	Differences number with GenBank sequence of	
	Phenotype by culture and other methods	Genotype (Accession no. of GenBank)		Miss matching	Genetic gaps
1	*M. canis*	*A. otae* (JX122197.1)	688/688 (100)	_	_
2	*M. canis*	*A. otae* (JX122197.1)	688/688 (100)	_	_
3	*M. canis*	*A. otae* (JX122197.1)	688/688 (100)	_	_
4	*M. canis*	*A. otae* (JX122197.1)	688/688 (100)	_	_
5	*M. canis*	*A. otae* (JX122197.1)	684/688 (99.4)	4	_
6	*M. audouinii*	*A. otae* (JQ922454.1)	356/383 (93)	23	1 insertion and 3 deletions
7	*M. audouinii*	*A. otae* (JX122197.1)	102/107 (95.3)	5	_
8	*M. canis*	*A. otae* (JX122189.1)	282/312 (90.3)	12	18 insertions
9	*M. canis*	*A. otae* (JX122189.1)	379/414 (92)	27	5 insertions and 3 deletions
10	*M. canis*	*A. otae* (JX122197.1)	679/689 (99)	7	2 insertions and 1 deletion
11	*M. canis*	*A. otae* (JX122197.1)	174/177 (98.3)	3	
12	*M. ferrugineum*	*A. otae* (JX122198.1)	653/662 (99)	4	3 insertions and 2 deletions
13	*M. ferrugineum*	*Arthroderma* sp. (FJ645724.1)	250/333 (75)	57	12 insertions and 14 deletions

Table 2: Show isolates of *Microsporum* spp. identification by phenotypic and genotypic methods

The difference of all strains which have not matching 100% identity with *A. otae* of reference strains sequence in GenBank Database demonstrated either from miss matching or genetic gaps (table, 2). Miss matching differences were demonstrate of three isolates sequenced, strain 5 with a 4-bp, strain 7 with a 5-bp and strain 11 with a 3-bp miss matching difference. While the remaining of the clinical isolates were demonstrated by miss matching as well as genetic gaps with insertion (strain 8) or deletion (strain 6) or both (strain 9, 10, 12 and 13).

10-Discussion

In this study An overall of 50 clinical fungal isolates of *Microsporum* spp. were taken from patients were affected 72% with tinea corporis, 24% with tinea capitis and 4% with tinea faciei. The results appeared that three species that phylogenetically closely related in the *A. otae* complex, *M. canis* (58%), *M. audouinii* (34%) and *M. ferrugineum* (8%). These species especially *M. canis* are a main causes of tinea capitis and tinea corporis [3]. [16] were isolated both *M. canis* (9.09%) and *M. audouinii* (1.51%) from tinea corporis, tinea capitis and tinea faciei. The infection by *Microsporum* spp. can be occurred from contact with any animals, especially cats and dogs or by contact with an infected persons or their fomite [20] [21] .

The identification of *Microsporum* spp. are usually depending on phenotypical features, especially *M. canis* by macroscopic and microscopic characteristics, in addition to biochemical tests. The phenotypical features of dermatophytes may predominatingly show variations, which oftentimes complicate the diagnosis, these alternation may be affected by numerous factors such as temperature, media, host and the user therapy [22]. All *Microsporum* spp. which isolated of this study were diagnosed of macro- and micromorphological characteristics according to several references [23] [24] [9]. [17] were identified four types of *Microsporum* spp. colonies based on culture characteristics in Sabouraud‘s dextrose agar and Potato‘s agar. Identification of some der- matophytes species requires special skills for reliable diagnosis, particularly in species with a degenerate appearance such as *M. ferrugineum* [25] [26].

In the present study, it was shown that there was some difficulty in demonstrating the phenotypic characterization of closely related isolates of *Microsporum* spp. and these difficulties may lead to hampering the diagnosis of closely related strains, which could avail for answering certain epidemiologically such as origins of infection or strains of a specific geographical area [27]. However, the phylogenetic relationship of species or strains sequences cannot be fully defined by conventional PCR methods [10]. So many studied have resorted to the use of the nucleotide sequencing in drawing the phylogenetic tree for clinical fungi and alignment analysis of nitrogenous bases sequencing or other modern methods where these meth- ods highlight the results of the rapprochement among fungal strains [21] [28] [29] [30] .

Our results appeared phylogenetic tree was showing a high sequence similarity in the ITS region of rDNA between 99.9-100% similarities for the isolates belong to the cluster 1, but this group with cluster 2 and 3 showed similarity of about 99.37% (fig. 5). By using Mega 6 software and display toggle conserved sites at 50% level with different colors, sequencing analysis of thirteen *A. otae* isolates showed identification of the colors in the nitrogenous bases alignment belong to the same species of the telomorph (fig. 6). Moreover, when using ITS region analysis and identity with reference strains sequence of GenBank Database (table, 2), possible discrimination that isolates showed a 100% identity with reference strains (strains 1-4), of isolates that were less identity which contained the mutations either from miss matching or genetic gaps (strains 5-13).

[31] were evaluated the taxa validity of 21 isolates of *M. canis* complex by phenotypic methods and compared with results of ITS region sequencing and amplified fragment length polymorphism (AFLP) analysis. [26] were differentiated among three species of *A. otae* complex and [32] were differentiated between *Trichophyton tonsurans* and *T. equinum* and in both studies used nucleotide sequencing of three of genetic loci ITS1, *BT2* and *TEF1* and determined SNP among species studied. The sequence alignment in the ITS2 region in the *Microsporum canis* complex (*M. canis*, *M. audouinii* and *M. fer- rugineum*) was observed nucleotide differences of many positions (deletion or insertion) and showed high similarities (94-100% similarities) among members of the complex [12]. [33] were differentiated among *Microsporum canis*, *Arthroderma otae* and *T. mentagrophytes*, the phylogenetic tree for these species occurred in neighbored clusters.

In conclusion, the present study confirmed that ITS region of rDNA has a significant effect on interspecies variation among strains belong to *Arthroderma otae* complex by using modern technique. The sequencing detected several mutations in multiple locus of these isolates either by miss matching or genetic gaps, enabling us to distinguish them suitably.

References

[1] C. Cafarchia, R. Iatta, M. S. Latrofa, Y. Graser, and D. Otranto, "Molecular epidemiology, phylogeny and evolution of dermatophytes," *Infection, Genetics and Evolution*, vol. 20, pp. 336–351, 2013. [Online]. Available: :

[2] M. Refai, A. E. Heidy, and E. Mahmoud, "Monograph on dermatophytes," *Department of Microbiology, Faculty of Veterinary Medicine, Cairo University*, vol. 75, 2013.

[3] Y. Graser, J. Scott, and Summerbell, "R.(2008).the new species concept in dermatophytes - a polyphasic approach. mycopathologia 1-18."

[4] H. D. Roqie, R. Vieira, S. Rato, and M. Luz-Martins, "Specific primers for rapid detection of microsporum audouinii by pcr in clinical samples," *J. Clin. Microbiol*, vol. 44, no. 12, pp. 4336–4341, 2006.

[5] "Rapid pcr based identification of two medically important dermatophyte fungi, microsporum canis and trichophyton tonsurans," *Acta Biologica Szegediensis*, vol. 53, no. 1, pp. 51–54, 2009.

[6] H. C. Li, J. Bouchara, M. M. Hsu, R. Barton, and T. C. Chang, "Identification of dermatophytes by an oligonucleotide array," *J. Clin. Microbiol*, vol. 45, no. 10, pp. 3160–3166, 2007.

[7] A. S. Shehata, P. K. Mukherjee, H. N. Aboulatta, A. I. E. Akhras, S. H. Abbadi, and M. A. Ghannoum, "Single-step pcr using (gaga)4 primer: utility for rapid identification of dermatophyte species and strains," *J. Clin. Microbiol*, vol. 46, no. 8, pp. 2641–2645, 2008.

[8] B. K. Jha, S. M. Murthy, and N. L. Devi, "Molecular identification of dermatophytosis by polymerase chain reaction (pcr) and detection of source of infection by restricted fragment length polymorphism," *(RFLP). J. College of Medical sciences-Nepal*, vol. 8, no. 4, pp. 7–15, 2012.

[9] C. Dhieb, B. Essghaier, D. E. Euch, and N. Sadfi-Zouaoui, "Phenotypical and molecular characterization of microsporum canis strains in north- tunisia," *Polish J. Microbiol*, vol. 63, no. 3, pp. 307–315, 2014.

[10] K. Makimura, Y. Tamura, T. Mochizuki, A. Hasegawa, Y. Tajiri, R. Hanazawa, K. Uchida, H. Saito, and H. Yamaguchi, "Phylogenetic classification and species identification of dermatophyte strains based on dna sequences of nuclear ribosomal internal transcribed spacer 1 regions," *J. Clin. Microbiol*, vol. 37, no. 4, pp. 920–924, 1999.

[11] Z. K. Imran, "Candida albicans ssp. dubliniensis stat.et comb. nov., a new combination for candida dubliniensis based on genetic criteria," *African J. Microbiology Research*, vol. 9, no. 17, pp. 1205–1214, 2015.

[12] H. C. Li, J. Bouchara, M. M. Hsu, R. Barton, S. Su, and T. C. Chang, "Identification of dermatophytes by sequence analysis of the rrna gene internal transcribed spacer regions," *J. Med. Microbio*, vol. 57, pp. 592–600, 2008.

[13] "Diagnosis of common dermatophyte infections by a novel multiplex real-time polymerase chain reaction detection/ identification scheme," *Clin. And Laboratory investigations*, vol. 157, pp. 681–689, 2007.

[14] G. A. Almola, Z. H. Al-Zubaidi, and Z. K. Imran, "Molecular characterization of mycovirus in the dermatophyte and non-dermatophyte fungi," *International J. Innovation and Applied studies*, vol. 10, no. 4, pp. 1073–1075, 2015.

[15] P. Kannan, C. Janaki, and G. S. Selvi, "Prevalence of dermatophytes and other fungal agents isolated from clinical samples," *Indian J. Medical Microbiology*, vol. 24, no. 3, pp. 212–215, 2006.

[16] K. M. A. Hasan and M. K. AL-Shibli, "The effect of risk factors and etiology on the distribution of clinical cases with dermatomycoses," *International J. Innovation and Applied studies*, vol. 14, no. 2, pp. 581–586, 2016.

[17] R. S. N. Brilhante, M. F. G. Rocha, R. A. Cordeiro, S. H. B. Rabenhorst, T. B. Granjeiro, A. J. Monteiro, and J. J. C. Sidrim, "Phenotypical and molecular characterization of microsporum canis strains in north-east brazil," *Journal of Applied Microbiology*, vol. 99, pp. 776–782, 2005.

[18] R. S. N. Brilhante, R. A. Cordeiro, J. M. F. Gomes, J. J. C. Sidrim, and M. F. G. Rocha, "Canine dermatophytosis caused by an anthropophilic species: molecular and phenotypical characterization of trichophyton tonsurans," *Journal of Medical Microbiology*, vol. 55, pp. 1583–1586, 2006.

[19] Z. K. Imran and Z. W. Alshammry, "Molecular diagnosis of candidemia of intensive carwe unite patients based on sequencing analysis of its regions," *International J. Pharm Tech Research*, vol. 9, no. 12, pp. 658–668, 2016.

[20] C. Romano, L. Valenti, and R. Barbara, "Dermatophytes isolated from asympomatic stray cats," *Mycoses*, vol. 40, no. 11-12, pp. 471–472, 1997.

[21] R. Sharma, S. Hoog, W. Presber, and Y. Graser, "A virulent genotype of microsporum canis is responsible for the majority of human infections," *J. Medical Microbiology*, vol. 56, pp. 1377–1385, 2007.

[22] D. Liu, S. Coloe, R. Baird, and J. Pedersen, "Application of pcr to the identification of dermatophyte fungi," *J. Med. Microbiol*, vol. 49, pp. 493–497, 2000.

[23] D. T. Lakshmipathy and K. Kannabiran, "Review on dermatomycosis: pathogenesis and treatment," *Natural Science*, vol. 2, no. 7, pp. 726–731, 2010.

[24] R. M. Beraldo, A. K. Gasparoto, A. M. Siqueira, and A. L. T. Dias, "Dermatophytes in household cats and dogs," *R.bras. Ci. Vet*, vol. 18, no. 2/3, pp. 85–91, 2011.

[25] T. D. Baere, R. C. Summerbell, B. Theelen, T. Boekhout, and M. Vaneechoutte, "Evaluation of internal transcribed spacer 2- rflp analysis for the identification of dermatophytes," *J. Med. Microbiol*, vol. 59, pp. 48–54, 2010.

[26] A. Rezaei-Matehkolaei, K. Makimura, G. S. Hoog, M. R. Shidfar, K. Satoh, M. J. Najafzadeh, and H. Mirhendi, "Multilocus differentiation of the related dermatophytea microsporum canis, microsporum ferrugineum and microsporum audouinii," *J. Med. Microbiol*, vol. 61, pp. 57–63, 2012.

[27] N. Sahgal and N. Magan, "Fungal volatile fingerprints: discrimination between dermatophyte species and strains by means of an electronic nose," *Sensors and Actuators B. Chemical*, vol. 131, no. 1, pp. 117–120, 2008.

[28] S. D. Respinis, M. Tonolla, S. Pranghofer, L. Petrinis, O. Petrinis, and P. P. Bosshard, "Identification of dermatophytes by matrix- assisted laser desorption/ionization time-of-flight mass spectrometry," *Medical Mycology*, vol. 51, pp. 514–521, 2013.

[29] K. M. A. Hasan, "Genotypic identification of cychloheximide resistance yeasts isolated from clinical cases with superficial mycosis," *International J. Pharm Tech Research*, vol. 9, no. 12, pp. 2455–9563,2016.

[30] Z. K. Imran and Z. A. Al-Karrem, "Evalution natural cloning of azole- resistant genes cdr1, cdr2, mdr and erg11 between clinical and soil isolates of candida albicans based on gene expression," *International J. Pharm Tech Research*, vol. 9, no. 11, pp. 229–236, 2016.

[31] Y. Graser, A. F. A. Kuijpers, M. E. Fari, W. Presber, and G. S. D. Hoog, "Molecular and conventional taxonomy of the microsporum canis complex," *Medical Mycology*, vol. 38, pp. 143–153, 2000.

[32] A. Rezaei-Matehkolaei, K. Makimura, G. S. D. Hoog, M. R. Shidfar, K. Satoh, M. J. Najafzadeh, and H. Mirhendi, "Discrimination of trichophyton tonsurans and trichophyton equinum by pcr-rflp and β-tubulin and translation elongation factor 1-α sequencing," *Med. Mycolo*, vol. 50, pp. 760–764, 2012.

[33] Z. K. Imran, S. M. A. Wtwt, and O. K. Hussein, "Molecular typing of dandruff pathogens and evaluated the antifungal activity of plant extracts," *International J. Pharm Tech Research*, vol. 9, no. 12, pp. 669–679,2016.