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Molecular Identification of *Microsporum canis* Isolated from Infected Children with Tinea corporis and Tinea capitis in Baghdad

Atyaf Saied Hamied[1*], Qusay Alnedawy[2]
[1] *Department of Biology, College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad, Baghdad, Iraq*
[2] *Ministry of Education, Baghdad, Iraq*

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Abstract

Microsporum canis is considered one of the filamentous fungi that cause surface fungal contagion in the humans and animals. The present study aimed to diagnose *M. canis* via the molecular method and differentiating its local Iraqi isolates from global isolates. Microscopic examination showed 55 specimens with *M. canis* from 130 specimens collected from children aged between 4-10 years suspected of dermatophytes who attended Medical City Laboratories and Baghdad Hospital in Baghdad city from 1/12/2022 to 1/3/2023. The results showed that the frequency of *M. canis* infections was 55/130 (42.31%). The results demonstrated significant differences in the animals' contact (p <0.0001), lesions (0.03) and habitation area (p =0.002). Whilst the ages appeared with non-significant differences (p =0.6). In order to confirm the microscopic examination and compare the Iraqi isolates with other global ones, the 55 positive results with *M. canis* were further diagnosed by using internal transcribed spacer (ITS) 1 and 4 universal primers with a size of 550 bp for PCR amplicons. PCR amplicons sequencing showed only one isolate of *M. Canis* that differed from global isolates registered in the database of NCBI. The Iraqi local isolate of *M. canis* was registered with accession number: OM185328. In conclusion, the PCR technique using ITS rDNA aided in confirming the detection of dermatophytes.

Keywords: Dermatophytes, *ITS* gene, *M. canis*, Tinea corporis, Tinea capitis

الكشف الجزيئي لـ *Microsporum canis* المعزولة من الأطفال المصابين بـ سعفة الجسم و سعفة فروة الرأس في بغداد

اطياف سعيد حميد [1*]، قصي النداوي [2]
[1] قسم علوم الحياة, كلية التربية للعلوم الصرفة–ابن الهيثم، جامعة بغداد، بغداد، العراق
[2] وزارة التربية، بغداد، العراق

الخلاصة

تعتبر *Microsporum canis* أحد الفطريات الخيطية التي تسبب العدوى الفطرية في الإنسان والحيوان. هدفت الدراسة الحالية إلى تشخيص *M. canis* بطريقة الفحص المجهرى و الطريقة الجزيئية و مقارنة العزلة العراقية المحلية من *M. canis* بالعزلات العالمية.تم جمع 130 نموذج من أطفال لديهم اعراض الإصابة الفطريه الجلديه تراوحت أعمارهم بين (4–10) سنة من كلا الجنسين الذين ارتادوا مختبرات مدينة الطب

[*]Email: atyaf.s.h@ihcoedu.uobaghdad.edu.iq

ومستشفى بغداد فى مدينه بغداد للفترة ما بين (1/12/2022 الى 1/3/2023)، أظهر الفحص المجهري ان 55 عينة مصابة بفطر *M. canis* من أصل 130 حالة يشتبه بإصابتها بالفطريات الجلدية. أظهرت النتائج اختلافات معنوية عند ملامسة الحيوانات (p <0.0001) ، وعدد القرح (0.03) ، ومنطقة السكن (p = 0.002) بينما ظهرت فروق غير معنوية بالنسبة للعمر(p = 0.6). لغرض مقارنة العزلات العراقيه بالعالميه وتاكيد التشخيص المجهرى تم فحص 55 عينة من النماذج الموجبةل *M. canis* بالفحص الجزيئى باستخدام بادئات لمنطقة ITS (ITS1 وITS4) بحجم 550 قاعدة نايتروجينية لناتج PCR. ظهر تسلسل القواعد النتروجينية لعزلة واحدة من *M. canis* تختلف عن العزلات العالمية المسجلة في قاعدة بيانات NCBI. تم تسجيل العزلة المحلية العراقية من *M. canis* برقم الدخول: OM185328. أستنتج من ذلك أن تقنية تفاعل البوليميراز المتسلسل باستخدام جين ITS كانت أكثر دقة في الكشف عن الفطريات الجلدية.

1. Introduction

Microsporum canis is considered one of the filamentous fungi which cause surface fungal infections in humans and animals [1]. It is the common agent that causes dermatophytes infection in household cats and in humans it causes tinea capitis [1, 2]. Dermatophytes attack and grow on the keratinous tissues such as skin, nails and hair in animals and humans resulting in dermatophytosis [3]. *M. canis* is considered a zoonotic infection that is prevalent in cats. It can put veterinarians, owners and animal care workers at the risk of catching the infection. Although dermatophytosis is being mostly limited in the immunocompetent persons, the zoonosis and infectious nature of *M. canis*, and its tendency to cause infection in children and individuals underserved medically are classified as apprehension factors [4, 5]. Similar to human infection, cats' dermatophytosis appears in various forms [1]. Healthy cats appear with zones of circular baldness and likely erythematous edges and scale [6]. Cats with a synchronous infection such as upper respiratory infection and elderly cats suffer from acute disease with the prevalence lesion and general diseases [1].

Classical methods which depend on microscopic examination and the culture methods are mostly applied to diagnose *M. canis* dermatophytes infection. Nevertheless, those techniques have many limitations such as slow growth and decreased sensitivity [7]. At this time, new molecular techniques that are suitable to conquer these restrictions, such as increasing the sensitivity and specificity of diagnosing, and also reducing the cost and time required for the identification [8]. *M. canis* detection by molecular methods has been reported in a little number of literature that has used internal transcribed spacer (ITS) 1 sequences of ribosomal DNA, or gene of β-tubulin have been applied as markers [9]. The present study aimed to diagnose *M. canis* via molecular method and differentiate its local Iraqi isolates from global isolates.

2. Methods

2.1Sample Collection and Study Design

The cross-sectional study performed in Baghdad city, involved 55 isolates of *M. canis*collected from 130 specimens from children aged between 4-10 years with suspected dermatophytes (tinea corporis and tinea capitis) who attended Medical city laboratories and Baghdad Hospital in Baghdad city from 1/12/2022 to 1/3/2023. Approval was taken from patients before examination and sample collection. Samples were collected in hygienic conditions from the skin and hair of patients suspected of dermatophytosis. The lesions were disinfected with 70% ethanol. Scales skin and crusts were collected from the erythematous margins via scraping of the inflammation margins using a sterilized surgical blade, and then part of it was put on clean slides. Hair samples were obtained via epilator using sterilized forceps [10].

2.2 Mycological Examination

The samples were diagnosed by microscope to detect the existence of fungi in hair and skin using 10% potassium hydroxide (KOH) to digest and soften keratin tissues so that fungal elements can be seen clearly under the microscope, and then covered with a cover slide, flamed and then diagnosed undera light microscope.

Cultural examination of the specimens was performed by cultivating the scrubbed skin and hair on SDA (Sabouraud dextrose agar containingchloramphenicol 50mg/L and cycloheximide 500mg /L) and then incubated at 30°C for 10-15 days. Cultural growth remained for four weeks before being deemed a negative culture [11]. The positive cultures were examined both macroscopically and microscopically for fungal identification.

2.3 Molecular Method

2.3.1DNA Extraction of M. canis

About 1gm of fungal mycelium from fresh cultures on Sabouraud dextrose agar was separated and then ground with a pestle in the mortar using ice to cool. The genomic DNA was extracted using Zymo Research DNA MiniPrep kit /USA (Cat No: D6005).

2.4 Genetic Detection of ITS Region by PCR

ITS detection was carried out by conventional PCR method. The primers used in the present study were from IDT Company, Canada (Table 1). The final volume of PCR mixture of 25µl included Taq PCR PreMix from Intron, Korea (5 µl), 10 pmol of primers (1µl), DNA (1.5µl) then the final volume was completed to 25 µl with nuclease-free water. The conditions of thermo cycling were denaturation for 3 min at 94°C; 35 cycles for 45s at 94°C, 35 cycles for 1 min at 52°C; 1min at 72°C and 72°C for 7 min as the final incubation. A thermal cycler Gene Amp/PCR system 9700/Applied Biosystem was used. PCR amplicons were electrophoresed in agarose gel (1.5%) and then visualized with safe red stain. (Intron/ Korea) by using UV.

Table1: Sequence of Universal Primer ITS1 and ITS4 [12].

Gene Region	Primer	Sequence	Tm (°C)	GC (%)	Product Size
ITS1	Forward	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	550bp
ITS4	Reverse	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41 %	

2.5 The Sequencing of DNA and Phylogenetic Tree Analysis

PCR amplicons sequencing was performed by sequencer from Applied Biosystem Inc, Macrogen Korea. The results were analysed via BLAST program in NCBI, and the phylogenetic tree was analysed via the Neighbour Joining method. Evolutionary distances were determined via the Jukes-Cantor model for estimating phylogenetic distances designed in MEGA11 [13].

2.6 Statistical Analysis

The statistical analysis was conducted using MedCalc Software Ltd 2023. Chi-square was then applied and the results were considered significant if *p*-value greater than 0.05and non-significant if *p*-value was less than 0.05.

3.Results

3.1 Microscopic Examination

Microscopic examination revealed the presence of *M. canis* in 55 out of 130 cases suspected with dermatophytes. The colonies were woolly to cottony with a white to yellow in colour on the front side and yellow on the reverse side. The macroconidia appeared spindle-

shaped with an asymmetrical apical lump and were divided into 6-15 cells with dense outer cells and had a dense outer cell wall. Microconidia was pear-shaped to clavate.

3.2 Demographic Distribution

The current study demonstrated the frequency infection of *M. canis* as being 55/130 (42.31%). The results showed a significant difference in the animals contact (p <0.0001), lesions (0.03), and habitation area (p= 0.002). Whilst the ages 4-10 years appeared with non-significant differences (p= 0.6) (Table 2).

Table 2: Demographic data of *M. canis* infection

Demographic Data	Number of Investigated	Positive Case of *M. canis*	*p*-value
Ages (4-7)	40	15(37.5%)	0.6
(8-10)	90	40(44.4%)	
Habitation area			
Rural	110	35(31.82%)	0.002
Urban	20	20 (100%)	
Lesions			
One	15	1(6.7%)	0.03
More than One	115	54(46.96%)	
Animal Contact			
Yes	130	55(42.31)	<0.0001
No	0	0(0%)	

3.3 Genetic Detection

Genetic diagnosis for the 55 *M. canis* isolates was further diagnosed by using ITS universal primers (ITS1 and ITS4) where it was amplified by PCR amplicons with 550 base pair for each specimen and then electrophoresed (Figure 1). Further PCR amplicons sequencing revealed that only one isolate of *M. canis* isolates was different from global isolates registered in database of NCBI. The Iraqi local isolate of *M. canis* in the current study was registered with accession number: OM185328 and showed a compatibility of 97-98% with isolates from Egypt, Russia, China, Kazakhstan, Thailand, India and Hong Kong when aligned with isolates in the gene bank. There were various positions substitution (transition and transversion) when aligning the local isolates with global isolates. Phylogenetic analysis for local isolate of *M. canis* showed convergent with isolate of Hong Kong, China, Kazakhstan, Thailand and India, while it was divergent from Egyptian and Russian isolates (Figure 2).

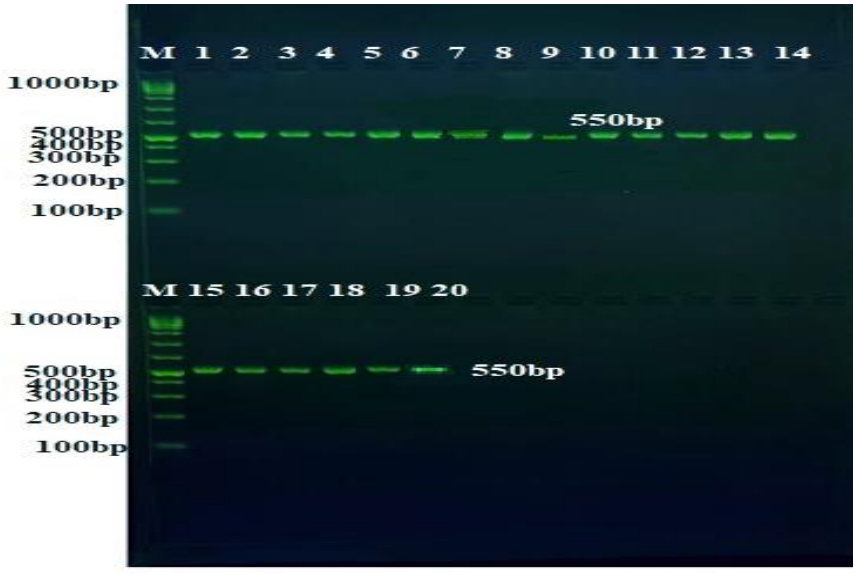

Figure 1: PCR amplicons of *ITS* gene (550bp) of some samples for *M. canis*. Electrophoresis in 1.5% agarose gel, M represents DNA ladder

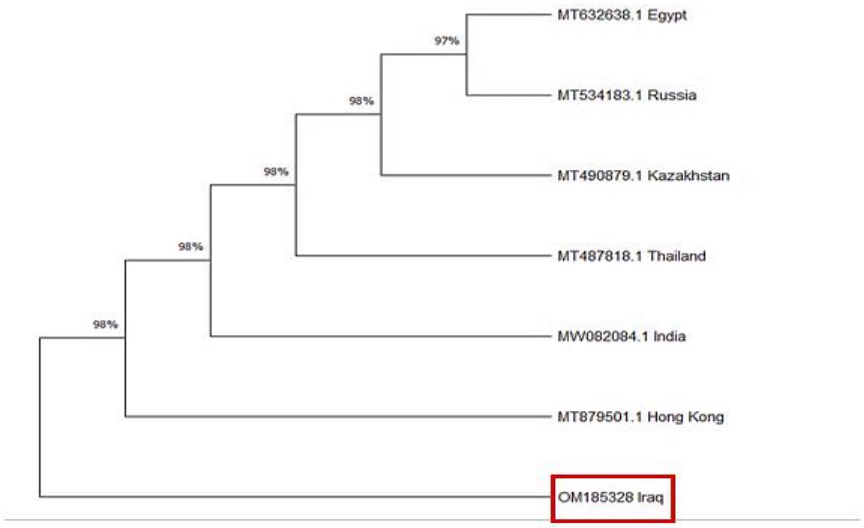

Figure 2: Phylogenetic tree analysis for the Iraqi isolate via the Neighbour-Joining method.

4. Discussion

Microsporum canis is the most prevalent causative agent tinea corporis and tinea capitis in the population. Dogs and cats are considered the normal hosts [9, 14]. One study in Iraq reported 14.63% prevalence of *M. canis* (24 out of 83 cases) [15]. Another Iraqi study showed a high frequency of dermatophyte infection [16]. It was also observed that at 83.33% the most prevalent dermatophytes species in dogs were *Microsporum canis* and *Trichophyton mentagrophytes* [17]. Moreover *Microsporum canis* was also detected in other domesticated animals such as cattle at 25% in Wasit city, Iraq [18]. In some other Iraqi studies, dermatophytes diagnosed in the population included 3 genera *Microsporum, Trichophyton,* and *Epidermophyton* [19].

In Egypt, Fayed, and El-Esawy [20] showed that the frequency of *M. canis* infection in children was more frequent at 42.9% than *T. mentagrophytes* (23.3%).

In the latest years, *M. canis* prevalence in humans has increased to a larger extent. Particularly, in children, *M. caniscanis* represents the common cause of infection [21]. Outbreak of infections in children with tinea corporis have been described in many studies [22]. The current study showed that cats and dogs represent the main host for *M. canis*, and those animals are more distributed in the community. Poor environmental sanitation also, particularly in the rural regions, represents the best environment for fungi. The fungi can exist for many months or years in sofas, chairs, beds, furniture, walls, floors, and things related to transportation, grooming, and places of animal housing [23]. Another study showed that *M. canis* distribution in children was associated with handling cats and dogs [24].

The appearance of clinical isolates in different phenotypes leads to complication in diagnoses as this species detection demands technical practice. Presently, the molecular techniques for fungal identification have become more common as they allow for final detection within 1 – 2 days with accurate sensitivity, paving the way for more suitable therapy in controlling the infection [8].

Amplification of ITS region was used for diagnosing fungi isolated during this study as it provides an appropriate taxonomic accuracy for most fungi. Moreover, there are a large number of sequences for this region in GenBank which permited comparison of the sequences that were obtained. Therefore, nucleotide sequence variation in this region can be used to classify the majority of fungi [12]. Where PCR assay was used to detect *M. canis* in patients' specimens, the hair of animals, and clinical samples in this assay appeared with 100% sensitivity and specificity [9].

PCR method can assist in the detection of dermatophytes more rapidly and precisely. High detection efficiency of dermatophytes using molecular techniques was observed in one of the Iraqi studies performed to detect dermatophytes *M. canis* and *T. mentagrophytes* in children with tinea capitis [25]. In USA, Moskaluk *et al.* [5] and Gharib *et al*. [26] showed the positivity of 191 samples for *M. canis* using the ITS-1 gene.

5.Conclusion

It can be concluded that using ITS primers in the PCR technique was more accurate in detecting dermatophytes and that the sequencing of ITS can help to differentiate local Iraqi isolates of *M. canis* from its global isolates when conducting alignment with other isolates in NCBI. Using phylogenetic analysis, the Iraqi local isolate has been registered with accession number: **OM185328.**

Conflict of Interest

There are no discrepancies of interest.

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