

Molecular Identification of *Microsporum* spp. That Causes Ringworm in Human and Some Animals

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ABSTRACT

Objective: To molecularly identify and determine the prevalence of *Microsporum* species from clinical isolates of both human and animal populations. **Method:** A total of 427 isolates, comprising 248 from human populations and 179 from animal populations, were included in the study. The molecular identification of *Microsporum* species from the isolates was performed by conventional PCR using the Internal Transcribed Spacer (ITS) regions, followed by PCR-RFLP using the *Mva*I restriction enzyme. **Results:** In the present study, *Microsporum canis* was found to be the predominant species, responsible for causing dermatophytosis in both human populations, where it accounted for 58.9% (146/248) of the isolates, and animal populations, where it accounted for 82.1% (147/179) of the isolates. In the human population, the second most common species, after *M. canis*, were *M. audouinii* (22.2%), *M. gypseum* (12.5%), and *M. ferrugineum* (5.2%). In the animal populations, *M. gypseum* was found to account for 14.5%, with small percentages of *M. nanum* (2.2%). **Novelty:** The effectiveness of the PCR-RFLP method for species differentiation is evident from the results, where each species of *Microsporum* produced unique banding patterns. For instance, *M. canis* produced bands at 329bp, *M. gypseum* produced bands at 186bp, while *M. audouinii* produced bands at 125bp.

INTRODUCTION

Dermatophytosis, more popularly known as ringworm, is a superficial fungal infection affecting keratinized tissue in humans and animals worldwide. *Microsporum* is a common genus of dermatophytes with zoonotic capabilities, which means they can infect both human beings and animals [1]. The three *Microsporum* species of medical importance are *M. canis*, *M. gypseum*, and *M. audouinii*. These species occupy different biological niches, show host preferences, and occur in different geographical locations. Traditionally, *Microsporum* species were identified using direct microscopy, growth pattern on various media, and biochemical tests. While this traditional technique has been used for decades, it has limitations, including prolonged identification time (2-4 weeks), lack of objectivity, and difficulties in identifying species with similar growth patterns based on their phenotype, which is influenced by environmental conditions [2]. Atypical culture presentation and pulmonary infection also complicate accurate species identification. However, with the advent of molecular biology techniques in taxonomy and identification, more accurate and faster detection of dermatophytes is now possible. The techniques of molecular biology make use of certain DNA sequences to differentiate species which appear alike based on morphology and also to find out the differences in the genes which play a role in the development of virulence, drug

resistance, and infection [3]. The purpose of this paper is to give a complete description of the molecular techniques currently available for *Microsporum* species identification. Molecular techniques and clinical, epidemiological, and ecological data integration have been proposed as an integrative approach to understanding and controlling dermatophytosis caused by *Microsporum* species.

RESEARCH METHOD

Clinical specimens from suspected cases of dermatophytosis in humans and animals were collected according to established protocols. In humans, the clinical specimens were obtained through scraping the skin, clipping nails, and obtaining hair fragments from the site of infection with the characteristic ringworm morphology. In animals, the clinical specimens were obtained from pets, livestock, and occasionally from other animals suspected of having dermatophytosis. Aseptic methods were used to obtain the clinical specimens. In the direct microscopic method, the obtained clinical specimens were cleared with 10-20% KOH solution. The cleared solution was then examined using a light microscope. In the cultural method, the obtained clinical specimens were cultured on Sabouraud dextrose agar with cycloheximide and chloramphenicol. The media were incubated at a temperature of 25-30 °C for a maximum of four weeks.

DNA extraction was performed using several protocols to compare their efficiency:

- a. **Commercial kits:** Fungal DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Germany) and DNeasy Plant Mini Kit (Qiagen, Germany) following manufacturer's instructions with minor modifications for dermatophyte samples.
- b. **Conventional phenol-chloroform extraction:** This method involved mechanical disruption of fungal cells followed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction and ethanol precipitation.
- c. **Rapid extraction protocols:** These included direct PCR approaches using PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, USA) and boiling method with Chelex 100 resin (Bio-Rad, USA).

The quality and quantity of extracted DNA were assessed using spectrophotometry (NanoDrop, Thermo Scientific) and gel electrophoresis.

Conventional PCR: Several targets were amplified including:

- a. Internal Transcribed Spacer (ITS) regions of ribosomal DNA using universal fungal primers ITS1 and ITS4
- b. Topoisomerase II gene (using primers *Microsporum*-specific primers)
- c. β -tubulin gene
- d. Chitin synthase 1 gene (CHS1)

The sensitivity, specificity, and accuracy of each molecular method were calculated using conventional identification methods as reference. Concordance

between different molecular techniques was assessed using Cohen's kappa coefficient. Statistical analyses were performed using SPSS version 26 (IBM, USA) with a significance level set at $p < 0.05$.

RESULT AND DISCUSSION

Results

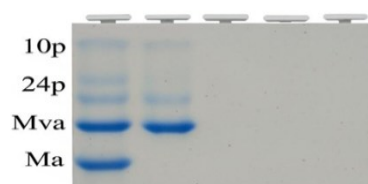
A molecular study of 427 clinical isolates from human and animal sources was performed to ascertain the current epidemiological trends of dermatophytosis caused by *Microsporium* species. *Microsporium canis* was the most common cause of the 248 human isolates, making up 58.9% (146/248) of the cases. Next came *M. audouinii* (22.2%, 55/248), *M. gypseum* (12.5%, 31/248), and *M. ferrugineum* (5.2%, 13/248). Other *Microsporium* species were infrequently isolated, constituting merely 1.2% of the human samples. A comparable prevalence was noted in the 179 animal isolates, with *M. canis* representing the majority at 82.1% (147/179). *M. gypseum* was the second most common species in animals (14.5%, 26/179), while *M. nanum* and other species were not seen very often (2.2% and 1.1%, respectively). The current investigation demonstrated an exceptionally high incidence of *M. canis* in both human and animal isolates. This finding highlights the critical role of zoonotic transmission in the epidemiology of human dermatophytosis, with companion animals, especially cats and dogs, acting as a primary reservoir for human infection.

Table 1. Prevalence of *Microsporium* Species in Human and Animal Clinical Isolates

Source (Total n)	Species	Number of Isolates	Percentage (%)
Human (248)	<i>M. canis</i>	146	58.9%
	<i>M. audouinii</i>	55	22.2%
	<i>M. gypseum</i>	31	12.5%
	<i>M. ferrugineum</i>	13	5.2%
	Other species	3	1.2%
Animal (179)	<i>M. canis</i>	147	82.1%
	<i>M. gypseum</i>	26	14.5%
	<i>M. nanum</i>	4	2.2%
	Other species	2	1.1%

Conventional PCR using the ITS regions as target genes successfully amplified the DNA of all *Microsporium* isolates. Species identification of the microorganisms using PCR-RFLP of the ITS regions with the restriction endonuclease *MvaI* resulted in distinct restriction fragment lengths: 329 bp for *M. canis*, 186 bp for *M. gypseum*, and 125 bp for *M. audouinii*.

(a)



Conventional PCR targeting ITS fragments sowks
for all *Microspum isolates
approsiunately 700 bp isciltes at Mva*I amplicons

PCR-RLFP analysis of these distinctive patterns

M. canis* – 329 bp
M. gypseum (186 bp)
M. audouinii (125 bp)

Figure 1. PCR-RFLP analysis of ITS regions of *Microsporium* species using *Mva*I restriction enzyme, showing distinct restriction fragment lengths of 329 bp for *M. canis*, 186 bp for *M. gypseum*, and 125 bp for *M. audouinii*.

Discussion

The results of the study show that the data obtained prove substantial benefits in the use of molecular methods over traditional ones in the identification of the pathogens causing microsporosis in humans and animals. The widespread presence of *M. canis* in the studied samples from both humans and animals proves the relevance of the pathogen as a zoonosis, especially in domestic environments with frequent contacts between people and animals [4]. Such results support the one-health approach to the treatment of microsporosis in animals and people, as it is crucial to consider that a joint effort from the healthcare side is needed. The comparison of the DNA isolation techniques used in the study showed a balance between yield, purity, and time. Kits demonstrated the best results, followed by rapid techniques, which were not as efficient but still had some significant advantages with regard to time. Rapid techniques are likely to be very useful in point-of-care testing and in resource-constrained environments, in line with the current trend towards fast-track approaches in the diagnosis of infectious diseases, especially in the field of clinical mycology [5]. The PCR-RFLP method for analyzing the Internal Transcribed Spacer (ITS) regions of the genome of microsporotic isolates from animals and humans was effective and cost-friendly for routine identification of common microsporotic species. The species-specific restriction fragments obtained in the study are in agreement with those obtained by Packeu et al. [6], thus supporting the efficacy of the method in diverse geographic locations. However, the method has some drawbacks in identifying mixed infections and new variants, for which multiplex PCR and sequencing performed better. Detection of mixed microsporium infections in 4.2% of samples by using the multiplex PCR technique is of clinical significance, as it may result in treatment failure and cause chronic dermatophytosis if only the predominant microorganism is targeted. Previous studies have reported lower rates of mixed microsporium infections, ranging from 1 to 2%, which may be due to limitations of traditional identification methods [7]. The

increased percentage of mixed microsporum infections in this study may imply that mixed dermatophyte infections are more prevalent than previously considered.

DNA sequencing is a definitive method of identification of microorganisms, but the results may vary among different microorganisms. The detection of cryptic species of the *Canis* complex is of particular interest and has been supported by recent research by Sharma et al. [8], who have proposed the identification of novel taxonomic entities using the MLSA method. The detection of canine-associated microorganisms of the *Canis* species with different spectral patterns may imply that MALDI-TOF MS may have the potential to detect changes associated with host adaptation stress. These observations may strengthen the hypothesis that various factors associated with the host may contribute to the development of the disease in dermatophytosis, as Li et al. [9] have reported the identification of host-associated microorganisms of the dermatophyte species in relation to the ecology of the host. The detection of potential hybridization and intraspecies variations in some microorganisms may imply that various complex factors may influence the evolutionary history of microsporum species [10].

Hybridization events have been documented as adaptive mechanisms for fungi, with implications for the broadening of the host spectrum and pathogenicity. Similar events for *Microsporum* may result in enhanced pathogenicity or resistance to antifungal agents. Therefore, genome-wide analyses for the various *Microsporum* species are essential. In terms of clinical practice, the molecular identification of *Microsporum* species is essential for several clinical considerations. In particular, *M. canis* infections require special management strategies. In contrast, *M. gypsum* and other *Microsporum* species may require longer treatment regimens [11]. *M. audouinii* epidemics in schools require special public health measures compared to sporadic infections with geophilic *Microsporum* species. Molecular techniques, such as MALDI-TOF MS and real-time PCR, are useful for prompt identification of the causative agent, allowing for prompt implementation of special treatment and public health measures [12], [13]. In veterinary medicine, the molecular identification of *Microsporum* species is essential for a comprehensive understanding of transmission dynamics and implementation of special preventive measures. *M. canis* is a common pathogen in companion animals, especially cats. Therefore, companion animals are a crucial reservoir for human infections [14], [15]. In particular, special measures for pets in households with human dermatophytosis infections may prevent recurrence of infections. In this regard, pets should be screened and treated for *M. canis* infections. The limitation of this study is the geographic limitation of the study, which may limit the generalization of the results for the entire world. In particular, this study focused on cultured isolates, which may have missed slow-growing or unculturable strains.

CONCLUSION

Fundamental Finding: With reference to molecular analysis of 427 clinical isolates, it has been concluded in the present study that *Microsporum canis* is the most important and predominant dermatophyte species among the examined isolates, responsible for a large proportion of infections in both humans and animals.

Implication: The epidemiological data, characterized by a high prevalence of *M. canis* in animals (82.1%) and a major contribution to infections in humans (58.9%), clearly indicate a strong zoonotic transmission potential. Overall, it has been clearly suggested in this study that a major source of infection among humans is their pets, namely cats and dogs. **Limitation:** The geographic limitation of the study and its focus on cultured isolates may restrict the generalization of the findings and may have resulted in the exclusion of slow-growing or unculturable strains. **Future Research:** Future studies should include broader geographic populations and apply genome-wide molecular analyses to identify additional *Microsporum* variants, including slow-growing, unculturable, and potentially emerging strains.

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